THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 137 NOVEMBER 1, 1942 No. 4

THE EFFECTS OF HEIGHTENED NEGATIVE PRESSURE IN THE CHEST, TOGETHER WITH FURTHER EXPERIMENTS UPON ANOXIA IN INCREASING THE FLOW OF LUNG LYMPH

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Received for publication June 22, 1942

In 1921 Graham suggested and showed experimentally that abnormally high negative pressure in the chest might cause pleural transudates, particularly if pulmonary edema was already present, fluid being sucked from the lung capillaries into the pleural sacs. Brock and Blair (1931), utilizing a heart-lung preparation, actually saw bloody fluid exude from the surface of the lungs under experimental conditions which permitted increased negative pressure within the glass chamber containing the lungs. Yamada (1933) made pleural punctures in several hundred Japanese soldiers. When the puncture followed a period of rest he obtained small amounts of fluid in 29 per cent of his subjects. If, however, the same individuals exercised vigorously, thus going through a period of violent breathing, Yamada obtained fluid on puncture in 70 per cent of his subjects. He did not require the men to breathe against resistance, that is, to inspire through a tube containing cotton wool or in some way arranged to make inspiration difficult. That situation, coupled with work sufficient to produce deep breathing or with inhalation of 5 to 10 per cent carbon dioxide mixed with air or oxygen to accomplish the same purpose, would in all probability have produced even more marked results than those gained through exercise alone, since maximum degrees of negative intrathoracic pressure may be produced by such conditions.

In the first group of experiments which follows, the flow of lymph from the lungs and heart has been observed during breathing against abnormal inspiratory resistance.

EXPERIMENTS AND DISCUSSION. *Anatomical considerations.* In a previous paper Warren and Drinker (1942) described the collection of lymph from the lungs of dogs. They showed that lymph entered the right lymphatic duct from all parts of the lungs except the upper section of the left lung, and that this lymph in no case contributed materially to the lymph flow from the thoracic duct. This was a surprising finding to the authors, though to a degree described by anatomists for man (Sappey, 1874; Rouvière, 1932).

The facts of the situation for the dog are shown in figure 1. In the left semi-diagrammatic drawing, the heart, great vessels, and inflated lungs are shown as they would appear if the anterior wall of the chest were removed. So far as the lymphatics from the lungs are concerned, the superior vena cava is an important guide. If it is followed upward in the illustration, the right lymphatic duct is seen entering the right subclavian vein just above a fairly constant lymph node. Between the superior vena cava and the aorta, two or more nodes are found which lie upon the surface of the trachea. The lymphatics entering this chain carry lymph from the heart and lungs. In the drawing, the vessels to and from

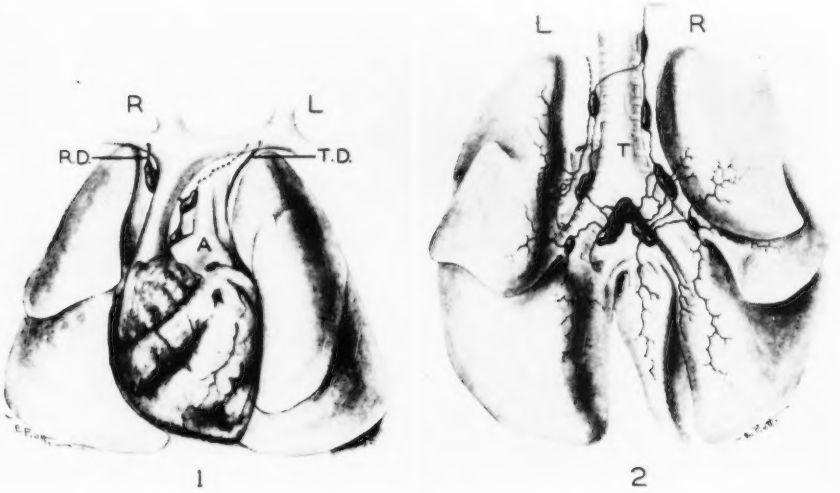

Fig. 1. The diagrammatic drawing (*1*) to the left shows the heart, lungs and great vessels of the dog viewed anteriorly. The superior vena cava is the prominent landmark. On the right, there is a single node not far below the subclavian vein. To the left of this vessel two other nodes are usually found, and from the upper an inconstant vessel may occasionally cross to the thoracic duct. The drawing (*2*) to the right illustrates the fact found in almost all cases that lung lymph drains ultimately to the right side. It is a rear view of the lymphatic system and lungs of the dog.

the nodes are single. As a matter of fact a number of afferent lymphatics enter each node, and with experience one can learn to distinguish the smaller cardiac lymphatic going to the upper node between the superior vena cava and the aorta, from several other afferent vessels carrying lymph from the lungs.

The right drawing (*2*, fig. 1) shows the gross features of lymph drainage from the lungs of the dog viewed from the rear. Drainage of lymph to the right is clearly evident. In both *1* and *2*, figure 1, a vessel is shown as a broken line. Such vessels represent infrequent connections, in *1* crossing from the right to the thoracic duct or to enter the subclavian vein independently, and in *2* ascending directly to join the thoracic duct. Neither of these connections is large, and if

present they are unimportant since the lymph diverted through them from the right duct cannula is insignificant in amount.

Many dissections have shown two things. First, in about three out of every six dogs the right lymphatic duct contains chyle. Sometimes the duct may be as large or larger than the thoracic duct, and is the main avenue for delivery of fat from the intestine to the circulation. Happily this irregular state of affairs is readily detected if right duct and thoracic duct lymph are compared, and it is found that the former is a clear fluid with 2 to 3.5 per cent protein, few red cells, and varying numbers of lymphocytes, while in the latter there is the milky opacity, which the chyle provides, together with a considerable number of red cells and a higher percentage of protein. Obviously, when the right duct lymph contained chyle an experiment upon lung lymph could not be done unless the abdomen was opened and the thoracic duct ligated just below the diaphragm. This has not been done in the experiments which follow.

These facts make it possible to collect lymph which in flow and composition represents that coming from the lungs, and in such a preparation one obtains practically all the lung lymph plus lymph from the heart which, in the absence of anoxia, will not, however, change unless cardiac activity is increased or decreased markedly, contingencies against which it is possible to provide adequate control. Cannulation of the right lymphatic duct has been a difficult task and is accomplished satisfactorily in about one out of five animals. If one wishes lung lymph alone, there is as yet no way of collecting it short of opening the anterior mediastinum under artificial respiration and cannulating a lung lymphatic, the procedure followed in the experiments upon anoxia reported in the latter part of this paper.

Experiments in which intrathoracic negative pressure was increased. A dog was anesthetized with sodium barbital given intravenously. This barbiturate is preferable to nembutal when respiration must be kept constant. Under nembutal the animal tends steadily to emerge from anesthesia, and this is particularly disturbing in respiratory experiments since new injections of the anesthetic are invariably depressing for a short period. The right lymphatic duct was cannulated and produced clear chyle-free lymph. At the start, the animal breathed 100 per cent oxygen under conditions precluding any possibility of resistance to inspiration. It was possible, however, by turning a three-way valve to compel the animal to breathe pure oxygen through a tube containing cotton wool. The obstruction to inspiration was very great, the negative pressure in the chest measured by means of a needle thrust through the chest wall reaching 56 mm. Hg at the height of inspiration. Clearly the experiment imposed a very severe strain which the animal could not have endured for many minutes. There was an immediate increase in lymph flow, and the lymph at once began to show red cells. Prior to the onset of obstructed inspiration the lymph contained 50 red cells per cubic millimeter. In 7 minutes the red cell content of the lymph rose to 57,900 per cubic millimeter. During this period of difficult inspiration the systemic blood pressure fell from a very even level of 143 mm. Hg. to 112 mm. Hg.

It was thus apparent that a large increase in negative intrathoracic pressure

drew fluid from the lung capillaries into the lung parenchyma and, under the excessive strain imposed by this experiment, not only plasma but red cells left the lung capillaries to be returned to the circulation in the lung lymph. When the obstruction to inspiration was removed the red cell content of the lymph disappeared at once, an interesting commentary upon the rapidity with which non-

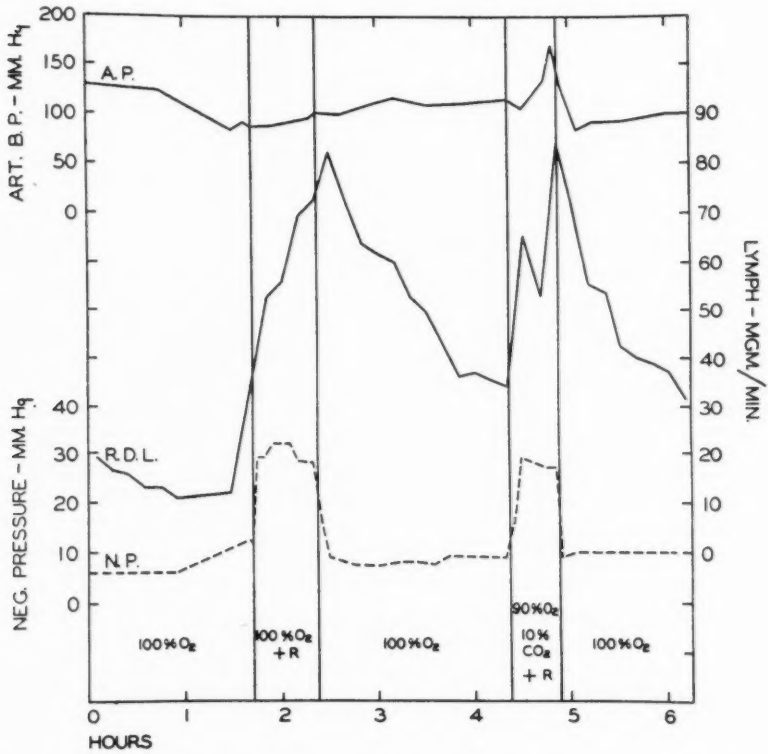

Fig. 2. The effect of breathing against inspiratory resistance. *Upper curve, A.P.*, arterial blood pressure; *middle curve, R.D.L.*, lymph flow in milligrams per minute; *lower curve (broken line), N.P.*, negative pressure in chest. During the first hour and a half lymph flow fell from nearly 20 to 11 mgm. per minute. Resistance to inspiration was then suddenly increased, and lymph flow rose at once to 82.5 mgm. per minute. When resistance was removed lymph flow fell to 34.0 mgm. per minute, but again increased sharply when the dog breathed 90 per cent oxygen and 10 per cent carbon dioxide with resistance.

ameboid cells reach avenues of lymphatic drainage in the moving, breathing lung.

Figure 2 summarizes the results of a more extended experiment. In this case a dog anesthetized with sodium barbital was prepared as follows. The trachea was cannulated in order to permit inhalation of oxygen or oxygen plus carbon

dioxide; the external jugular vein on the right side was made ready for introduction of a long glass tube passed down through the superior vena cava into the right auricle in order to obtain samples of mixed venous blood; blood pressure was taken from one femoral artery, and arterial blood samples under oil from the other. The right lymphatic duct was cannulated and found to produce chyle-free lymph.

There is little need to comment on the results as shown in figure 2. It is clear that increased negative pressure in the chest produces a huge increase in lymph flow. It may be contended that with the added respiratory effort there would be larger inflow into the heart and so more possibility of lymph production in the lungs. Cardiac output, determined by utilizing the direct Fick principle (Grollman, 1932), was 1.34 liters per minute prior to the first period of resistance to breathing, and at the height of the first period of resistance to inspiration rose to 1.42 liters per minute.

In the first period of resistance to inspiration, which is shown so dramatically in figure 2, certain other points deserve comment. First of all, during the entire experiment the oxygen content of the arterial blood did not fall below 20.80 volumes per cent. Knowing that anoxia is a potent cause of increase in flow of lung lymph, the animals in these experiments were given pure oxygen or 90 per cent oxygen plus 10 per cent carbon dioxide, so that anoxia should not enter the problem. A second point, already made, is that during the period of increased breathing cardiac output was practically unchanged. A third factor is the breathing itself when there is sudden resistance to inspiration. In this experiment, when the animal was compelled to breathe oxygen against resistance his minute volume fell to an average of 2.0 liters against 5.7 without resistance. It is clear from the figures upon blood oxygen that, owing to the use of 100 per cent oxygen for inhalation, anoxia did not occur. So far as can be seen, no essential factor affected lymph flow other than the increased negative pressure in the chest caused by breathing against resistance. There was no anoxia and cardiac output remained practically unchanged, but transudation occurred when the intrathoracic negative pressure was increased.

The experiments described thus constitute in the living animal a verification 1, of Graham's idea; 2, of the illuminating experiments of Brock and Blair, and 3, of the primitive but interesting observations of Yamada upon his docile Japanese soldiers.

In a second group of experiments lung lymph was collected during anoxia produced by breathing gas mixtures containing low amounts of oxygen.

In a previous paper (Warren and Drinker, 1942), it was shown that lymph collected from the lungs of dogs increased under such circumstances. In these animals the anterior mediastinum was opened, the upper part of the sternum being removed and a cannula introduced into one of the several lung lymphatics. The lymph collected does not represent the total outflow from the lungs, but it is a reliable cross-section of lymph drainage at any moment.

In this first paper (Warren and Drinker, 1942) it was brought out from the literature that anoxia does not increase cardiac output (Grollman, 1932; Doi,

1921), but no experiments were done in which cardiac output was actually measured when anoxia caused increase in lymph flow from the lungs. The point is far from academic. If, with anoxia, the output of the heart increases markedly, then a heightened production of lymph may mean nothing more than enhanced filtration of fluid from the lung capillaries as the result of a capillary bed, possibly widened, and subjected to at least a slight increase in pressure. If, on the other hand, anoxia is accompanied by greater flow of lung lymph and the cardiac output remains the same as in the control period, or falls, then, given absolutely constant lung movement during the entire experiment, it is fair to attribute increased lymph flow to increased permeability of the lung capillaries arising from anoxia.

Experiments on low oxygen. In dogs anesthetized with sodium barbital and under artificial respiration the anterior mediastinum was opened and a lung lymphatic cannulated. Systemic blood pressure was taken from the femoral artery with a mercury manometer. Rate of lymph flow was measured by collecting lymph for a given period of time into weighed tubes which were then reweighed. Cardiac output was determined by utilizing the direct Fick principle, the samples of mixed venous blood being taken through a glass tube introduced into the right auricle through the jugular vein.

Figure 3 will suffice to show the results obtained. In this case artificial respiration with air was used first. After the initial high lymph flow which invariably follows the procedure of cannulation of lung or other lymphatics, due to temporary obstruction during operative procedures, the lymph flow became very steady at about 9.7 mgm. per minute. The blood pressure, as is usual in such experiments, became constant, and cardiac output at the close of the period of ventilation with room air was 2.13 liters per minute. Ventilation was then shifted to a mixture of 13.5 per cent oxygen and 86.5 per cent nitrogen. Lymph flow and blood pressure remained unchanged, and the cardiac output was 1.92 liters per minute. After 1 hour and 35 minutes' administration of this mixture of gases, during which the arterial oxygen saturation fell from 16.08 to 9.48 volumes per cent, a second shift in ventilation was made, this time employing 10 per cent oxygen and 90 per cent nitrogen. Immediately the output of lung lymph rose, at first during a period when blood pressure was uniform with preceding values. The cardiac output, however, fell to 0.95 liter per minute and arterial oxygen saturation to 4.23 volumes per cent, when it became necessary to return to ventilation with room air. Obviously (fig. 3), as soon as this was done lymph flow from the lungs fell to normal level and was undisturbed in the final period of the experiment when a mixture of 10 per cent carbon dioxide and 90 per cent oxygen was used. During the recovery period, when 10 per cent oxygen plus 90 per cent nitrogen was replaced by room air, oxygen saturation of the arterial blood reached 14.53 per cent and cardiac output was 1.27 liters per minute.

This experiment and others of similar type indicate that the production of extravascular fluid in the lung parenchyma, which, in good part, is drained off as lung lymph, depends upon anoxia of the lung capillaries and not upon augmenta-

tions of pressure and flow of blood through the lungs. No one who approaches the problem of pulmonary edema can be surprised at this conclusion. The normal mammal can experience tremendous increases in pulmonary blood flow during exercise. The vascular bed in the lungs is so huge and so distensible that significant increases in pulmonary blood pressure do not occur unless return of blood to the left ventricle is impeded. If one obstructs the pulmonary veins in an animal with a normal heart, and then gives adrenin or ephedrine so as to drive

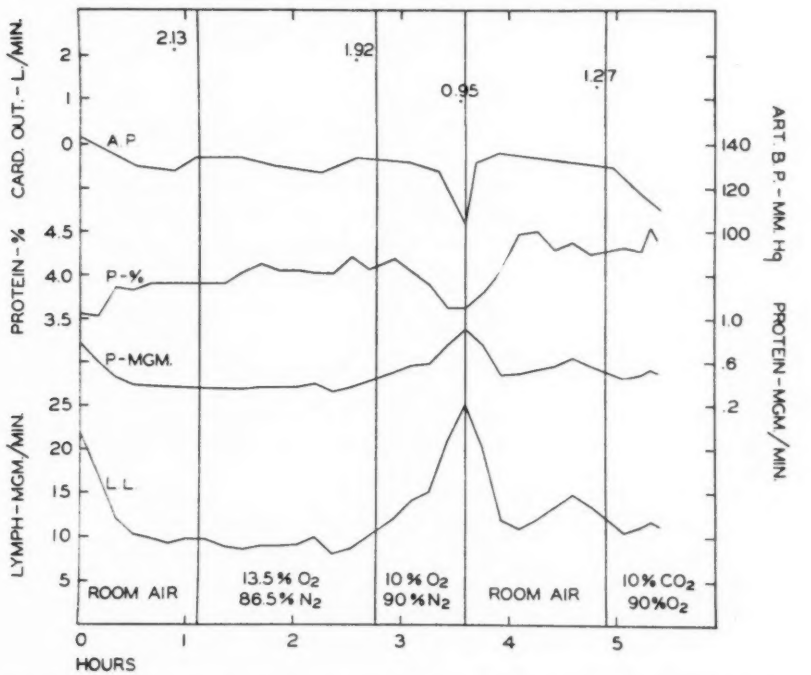

Fig. 3. Effect of lowering alveolar oxygen. *Dots with figures*, cardiac output in liters per minute; *upper curve*, *A.P.*, arterial blood pressure; *second curve*, *P-%*, protein in lung lymph in per cent; *third curve*, *P-MGM.*, protein in lung lymph in milligrams per minute; *fourth curve*, *L.L.*, milligrams of lung lymph per minute. *Ordinates*, as designated; *abscissae*, time in hours. Between the vertical lines, animal breathed gas mixtures as designated. Rate and stroke of respiratory pump uniform throughout.

up cardiac output, the pulmonary blood pressure can show short but surprising rises. In the experiment we have reported there has been no hindrance of return of blood to the heart through the lungs, and the output of the heart has fallen rather than risen during the period of anoxia. The conclusion is unescapable that oxygen lack alone has caused the lung capillaries to leak abnormally, and that as soon as extreme anoxia is relieved by ventilation with room air this increase in permeability ceases.

The effects of oxygen in maintaining the lung capillaries at normal permeability cannot be over-emphasized, and the subtlety with which fluid leaks from the capillaries into the lung parenchyma should be one of the ever-present nightmares of clinicians. If, to anoxia, as in cases of cardiac decompensation, increased pressure in the pulmonary capillaries is added, lung edema will occur with even greater rapidity and more malign results.

SUMMARY

1. The anatomy of the lung lymph drainage in the dog is described, and it is made clear that lung and heart lymph enter the blood through the right lymphatic duct.

2. It is held that cardiac activity, measured through output, remaining steady, fluctuations in lymph flow from the right duct reflect changes in the production and flow of lung lymph.

3. Experiments are described which show that when an animal breathes against resistance so as to increase intrathoracic negative pressure, the flow of lymph from the lungs increases; and if the resistance to inspiration is very high, there may be not only a gain in the flow of lung lymph but the fluid may actually become bloody.

4. These facts indicate the influence of the extravascular negative pressure in producing transudation of fluid from the lung capillaries into the lung parenchyma.

5. Further experiments are described in which, under absolutely uniform artificial respiration, expiration being without suction, dogs have been subjected to progressive severe anoxia.

6. When the oxygen in the mixture used for artificial respiration was 13.5 per cent, no increase in the flow of lung lymph was noted.

7. On reducing the oxygen to 10 per cent, a sudden increase in the flow of lung lymph occurred, which ceased promptly on returning to ventilation with air.

8. The changes in the production and flow of lung lymph during anoxia cannot be attributed to increased cardiac output or extension of the filtering bed of the lung capillaries, since the output of the heart fell markedly during anoxia. Consequently it must be concluded that when sufficient oxygen is not available the lung capillaries promptly become abnormally permeable, but if this condition is not allowed to go too far they readily return to normal by ventilation with adequate oxygen.

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VOLUME OF AIR MOVED BY ARTIFICIAL RESPIRATION IN ANESTHETIZED MEN

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Received for publication June 27, 1942

An earlier paper reported (1) results of the measurement of air moved by application of artificial respiration to conscious men. The primary purpose of that study was to evaluate the worth of a special method of artificial respiration, the Pole-top Method, by comparing its efficiency with the Schaefer Method. The latter method is designed for subjects lying prone. The Pole-top Method is designed for subjects in the trunk-vertical position and is of recent development (2) to meet a particular need, i.e., for application to men servicing electric power lines who may suffer "electric shock" and therewith respiratory paralysis as the result of contact with high voltage circuits.

These workers support themselves on the power-line poles by safety straps and when they are injured considerable time must elapse before they can be lowered to the ground. This time factor may well be of significance in the successful application of the older methods of artificial respiration. The Pole-top Method was developed to overcome this delay. It is applied by a fellow-worker who, supporting the victim on his own safety-strap in the trunk-vertical position (in effect holding the victim in his lap), spreads his hands over the lower abdomen and makes vigorous rhythmical upward lifts, thus forcing air to be expired. Further detailed description of the procedure may be found elsewhere (1, 2, 3).

Our earlier study, made on conscious men, indicated that the volume of air moved by the Pole-top Method when applied to subjects in the trunk-vertical position is somewhat greater than that moved by the Schaefer Method as applied to the same subjects in the prone position. Although we used only intelligent subjects presumably capable of relaxed passivity the results secured are open to criticism because the subjects may have co-operated unintentionally.

It thus seemed desirable to repeat the observations on anesthetized subjects in whom there was no possibility of co-operative aid. One of the barbiturates, sodium pentothal, was administered intravenously. The administration of this drug promptly effects unconsciousness and complete muscular relaxation. At the outset respiratory activity is depressed but it shortly returns to a fairly steady state which, by judicious administration of repetitive doses, may be safely maintained for a considerable period of time.

The data on the three subjects who were carried through the experimental procedure successfully are given in table 1. This comprises the detailed results secured when they were conscious as well as unconscious, together with their averages and the averages of the fifteen conscious subjects previously studied. Table 2 summarizes these results, the values being expressed in percentage in-

crease of air moved during application of artificial respiration over that of the corresponding control period of natural breathing.

TABLE 1

Respirations per minute and air moved per respiration in natural breathing and during artificial respiration applied before and after induction of anesthesia, together with the averages on fifteen conscious subjects previously reported

CONDITIONS	A 28 YRS. 139 LBS.	B 38 YRS. 160 LBS.	C 38 YRS. 167 LBS.	AVERAGE A, B, AND C	AVERAGE 15 SUBJECTS
Conscious					
Natural breathing in prone position:					
Resp. p. Min.	15	14	11	13	13
Cc. p. Resp.	375	625	770	590	545
Original Schaefer artificial respiration:*					
Resp. p. Min.	14	10	12	12	11
Cc. p. Resp.	424	1000	1111	845	868
Modified Schaefer artificial respiration:**					
Resp. p. Min.	13	11	10	11	11
Cc. p. Resp.	457	1250	1250	986	1028
Natural breathing in trunk-vertical position:					
Resp. p. Min.	14	16	10	13	15
Cc. p. Resp.	413	625	1000	679	558
Pole-top artificial respiration:					
Resp. p. Min.	10	14	10	11	10
Cc. p. Resp.	875	1111	1111	1032	1363
Unconscious					
Natural breathing in prone position:					
Resp. p. Min.	17	18	19	18	
Cc. p. Resp.	172	384	237	264	
Original Schaefer artificial respiration:*					
Resp. p. Min.	11	11	12	11	
Cc. p. Resp.	430	714	625	590	
Modified Schaefer artificial respiration:**					
Resp. p. Min.	9.5	10	12	10	
Cc. p. Resp.	571	910	768	746	
Natural breathing in trunk-vertical position:					
Resp. p. Min.	21	17	19	19	
Cc. p. Resp.	170	367	280	272	
Pole-top artificial respiration:					
Resp. p. Min.	11	12	13	12	
Cc. p. Resp.	714	912	770	799	

* Hands held in position between pressure strokes.

** Hands allowed to slip off at the end of each pressure stroke.

These findings, of course, are not strictly comparable to what might be expected to follow in individuals in whom all respiratory activity had ceased but they are probably as close as laboratory conditions can provide. They are fur-

thermore of interest in giving evidence of the relative efficiency value of the three methods of artificial respiration studied and, so far as we know, represent the first effort to evaluate the worth of artificial respiration in subjects under general anesthesia.

PROCEDURE. The procedure was much the same as in our earlier study, the same operator acting throughout, except the present subjects were not given preliminary training in the technique. The sequence of events was: 1. With mask and pneumograph in place the subject lay prone on the mat and rested five minutes. 2. Operator took position and waited two minutes. 3. Record of air moved in natural breathing. 4. Record of air moved by original Schaefer method (operator's hands held in position between pressure strokes). 5. Pause lasting two minutes. 6. Record of air moved by modified Schaefer method (operator's hands allowed to slip off at end of each pressure stroke). 7. Subject rose without displacing mask and assumed a trunk-vertical sitting position where he rested

TABLE 2

Raw data taken from table 1

Amount of air moved per minute and per respiration expressed as percentage increase over corresponding control period. The open figures represent the averages for the three subjects here reported; the bracketed figures represent the averages of the fifteen subjects previously reported. The right hand column of this table, as indicated by the heading, shows still another evaluation of the data.

	PERCENTAGE INCREASE OVER CONTROL PERIOD. AIR MOVED PER MIN.		PERCENTAGE INCREASE OVER CONTROL PERIOD. AIR MOVED PER RESPIRATION		PERCENTAGE DIFFERENCE IN AIR MOVED PER MINUTE BY ARTIFICIAL RESPIRATION WHILE ANESTHETIZED COMPARED TO NORMAL BREATHING WHILE CONSCIOUS
	Conscious	Anesthetized	Conscious	Anesthetized	
Original Schaefer artificial resp.....	32 (35)	37	43 (59)	123	15% decrease
Modified Schaefer artificial resp.....	41 (60)	57	67 (89)	183	3% decrease
Pole-top artificial resp.....	29 (63)	86	52 (144)	194	9% increase

five minutes. 8. Operator took subject onto his safety-belt strap followed by another pause of five minutes. 9. Record of air moved in natural breathing (trunk vertical). 10. Record of air moved by Pole-top method. 11. Anesthesia induced followed by pause until steady state of respiratory activity obtained. 12. Record of air moved by Pole-top method (trunk vertical, subject unconscious). 13. Unconscious subject lifted down without displacing mask and placed prone on mat and rested five minutes. 14. Operator took position. 15. Record of air moved in natural breathing (subject unconscious). 16. Record of air moved by original Schaefer method (subject unconscious). 17. Pause lasting two minutes. 18. Record of air moved by modified Schaefer method (subject unconscious).

RESULTS. The raw data from the observations are presented in table 1. It will be noted that the imposed respiratory rate (during artificial respiration) is less than in natural breathing while the amount of air moved per respiration is

greater. It is thus apparent that all three of the methods of artificial respiration employed are more than adequate to supply the requisite pulmonary ventilation.

In table 2 the foregoing data are used to express the results as percentage increases of the amount of air moved per minute and per respiration over the corresponding periods of natural breathing. Here the Pole-top Method shows as inferior to the Schaefer Method on the three new subjects when conscious. These values, compared with the values estimated from the conscious subjects earlier reported (1) (bracketed figures) suggest that a psychological factor may be involved in working with conscious subjects. Such a factor may, however, not make its appearance if the subjects are given preliminary training experience as was done in our earlier work. However, when the same three subjects were unconscious the values are clean cut and consistent.

It is, furthermore, to be noted in table 2 that in every instance the values, both for air moved per minute and per respiration, are greater when the subjects were unconscious.

Table 2 also shows (right hand column) the average percentage difference in air moved per minute by the three methods of artificial respiration while the subjects were unconscious compared with natural breathing while conscious. Anesthesia, of course, tends to depress the metabolism. Nevertheless it is of interest to note that the values presented are comparable to those shown in the other two columns of the table and tend to support the previous statement as to the relative efficacy of the methods tested.

We are indebted to Dr. W. H. Smith, Director of the Johns Hopkins Hospital, for facilities, and to the Duquesne Power & Light Co. for funds used in this study.

SUMMARY

Data are presented on the amount of air moved by artificial respiration in three subjects when conscious and when unconscious (anesthetized). These data indicate that: 1, more air is moved in the unconscious (anesthetized) than in the conscious subject with each (artificial) respiratory act; 2, the modified Schaefer Method (hands allowed to slip off at the end of each pressure stroke) is superior to the original Schaefer Method; 3, the Pole-top Method of artificial respiration, for the purpose intended, is an adequate and valuable procedure; 4, the results indicate that the Pole-top Method in the trunk vertical position is more efficacious than the Schaefer Method in the prone position.

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THE RÔLE OF THE ADRENAL CORTEX IN PREVENTING HYPOGLYCEMIC CONVULSIONS

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Received for publication June 27, 1942

The anti-insulin effect of hormones of the adrenal cortex and of the adrenotropic hormone of the anterior pituitary has been established by experiments of Grattan, Jensen and Ingle (1941) who showed that injection of these hormones may prevent insulin convulsions. However, these studies do not answer the question as to whether the physiological secretion of the adrenal cortex is adequate to modify the effects of insulin. The observation of Conn (1941) that hyperinsulinism in the human cannot be alleviated by injections of relatively large quantities of potent adrenocortical extracts suggests that the experiments of Grattan and collaborators are of a greater pharmacological than physiological significance. The experiments described in this paper were designed to throw some light on the physiological rôle of the adrenal cortex in insulin hypoglycemia.

METHODS. The experiments were performed on two groups of male rats (250 to 300 grams): one group was adreno-demedullated and the other was completely adrenalectomized. After a fast of 16 hours insulin (Lilly) was injected intraperitoneally and the blood sugar was determined after Hoffman (1937). The electroencephalogram (E.E.G.) was obtained by using Hoagland's method of inserting phonograph needles into the skull.

RESULTS. Table 1 shows the effects of 0.2 u. of insulin/kgm. injected intraperitoneally into adreno-demedullated and adrenalectomized rats. The lowering of the blood sugar is practically identical in both groups (table 1). There is, however, a marked difference in the frequency of cerebral symptoms as table 2 indicates. Symptoms in the form of coma or convulsions were observed in only 16 per cent of the adreno-demedullated animals although 76.5 per cent of the adrenalectomized animals showed these symptoms. The sensitivity of the latter group is further illustrated by eleven additional cases (not included in table 1) in which convulsions occurred so abruptly that blood samples could not be obtained. Inclusion of this group in table 2 raises the percentage of cerebral involvement to 84 in the adrenalectomized rats.

Since table 1 shows that convulsions do not depend solely upon the hypoglycemic level attained by the adreno-demedullated and adrenalectomized rats, it appeared to be likely that the rate of fall of the blood sugar might be the deciding factor. Comparison of the effect of insulin on 6 adrenalectomized and 9 adreno-demedullated rats shows that although the final blood sugar levels are similar the rate of fall is less rapid in the adreno-demedullated group.

It is worthy of note that the delayed fall of the blood sugar persisted when

[1] Aided by the John and Mary R. Markle Foundation.

insulin was injected into the adreno-demedullated rats in even greater amounts (0.3 to 0.5 u/kgm.) than were administered to the adrenalectomized group (0.2 u/kgm.). From these experiments it follows that the rate of fall of blood sugar is delayed by the hormones of the adrenal cortex. This effect of the adrenal cortex can be easily overcome in adreno-demedullated animals by injecting very large quantities of insulin. When 5 u/kgm. are injected, coma and convulsions occur in nearly all animals within 40 to 60 minutes. Apparently the insulin is circulating in the blood in concentration sufficiently high to offset the anti-insulin effects of the adrenal cortex. Therefore, the rate of the fall in blood sugar as well as the cerebral symptomatology becomes similar to that of adrenalectomized rats.

TABLE 1

Effect of 0.2 unit insulin (Lilly) intraperitoneally on the blood sugar of rats

NUMBER OF ANIMALS	TYPE OF OPERATION	INITIAL BLOOD SUGAR	BL. SUGAR AFTER 60 MIN.
		mgm.%	*mgm.%*
25	Adrenodemedullation	98 ± 4.5	74 ± 9.1
22	Adrenalectomy	96 ± 6.0	70 ± 9.9

TABLE 2

Cerebral symptoms following the injection of 0.2 u. insulin

NUMBER OF ANIMALS		TYPE OF SYMPTOMS	PERCENT OF SYMPTOMS
25	Adrenomedullated	2 comas 2 convulsions	16
33	Adrenalectomized	7 reflexes depressed 9 comas 12 convulsions	84

All of the data presented thus far indicate that the difference between the two groups with respect to cerebral symptoms cannot be explained on the basis of hypoglycemia alone. It seems of importance to prove this point more specifically. Table 3 shows how adreno-demedullated and adrenalectomized animals react at various hypoglycemic levels. It is obvious from the table that cerebral symptoms occur in adrenalectomized animals at relatively high blood sugar levels. None of the adreno-demedullated animals shows an involvement of the central nervous system at comparable levels. Moreover, at blood sugars between 66 and 58 mgm. per cent all adrenalectomized animals show coma or convulsions whereas in the adreno-demedullated groups 50 per cent of the animals remain normal.

This difference in the reactivity of the brain at low blood sugars is brought out in figure 1 in which the adrenalectomized animal reacts with coma characterized by the disappearance of alpha waves and the appearance of delta potentials.

The adreno-demedullated animal reaches a far lower degree of hypoglycemia without showing any change in E.E.G. or gross behavior.

TABLE 3

Relation of hypoglycemic level to cerebral symptoms

BLOOD SUGAR		BLOOD SUGAR	
I. Adrenodemedullated rats		II. Adrenalectomized rats	
mgm. %		*mgm. %*	
74	6 normal	77–80	6 normal 2 comas
70	13 normal	74	1 normal 1 depressed 1 coma
66	6 normal 1 depressed reflexes 2 comas	70	2 normal 4 depressed 1 coma
62	3 normal 1 depressed reflexes 1 coma	66	1 coma
58	1 normal 2 depressed reflexes 2 comas	62	5 comas 2 convulsions
		58	5 convulsions 1 coma

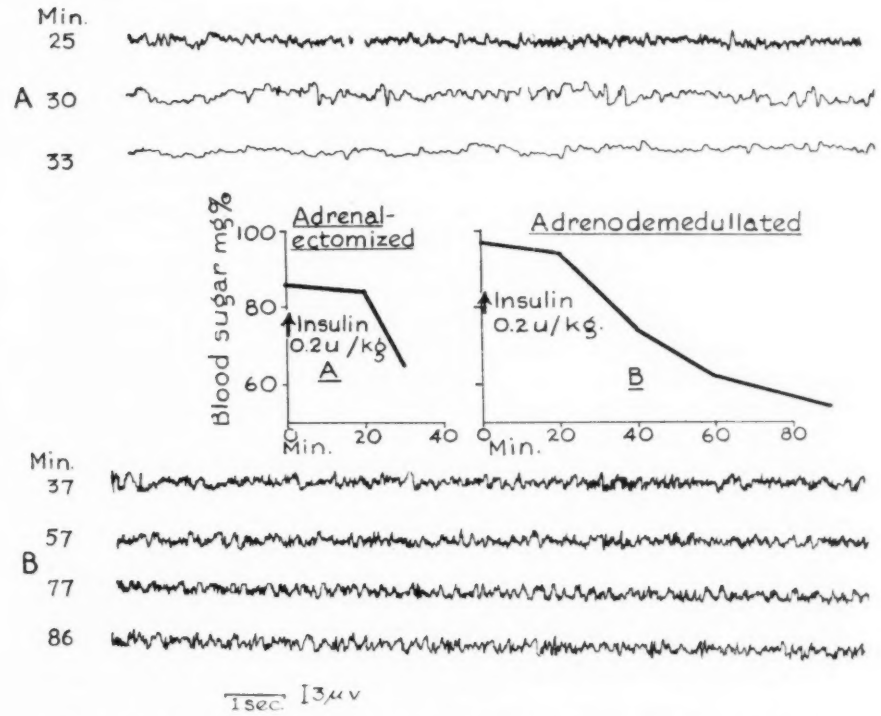

Fig. 1. A. Adrenalectomized rat; 0.2 u. insulin/kgm. intraperitoneally. Effect on blood sugar and E.E.G.

B. Adrenodemedullated rat; same as above.

Discussion. Our observations on cerebral symptoms in adrenalectomized and adreno-demedullated animals confirm similar studies of Zucker and Berg

(1937). However, the reactivity of the blood sugar is different in the experiments of these authors and our own. Zucker and Berg observed a delay in the recovery of the blood sugar of their adrenalectomized dogs but saw no differences in the rate of fall. It should be mentioned however that these authors used dogs and maintained the animals with adequate quantities of cortical extracts. Our adrenalectomized rats were not given any hormones but kept in good condition solely by the ingestion of 2 per cent NaCl.

Since in our earlier work (Gellhorn, Feldman and Allen, 1941) adreno-demedullated animals often showed coma when injected with 0.2 u. insulin/kgm. it seems probable that these animals suffered from adreno-cortical deficiency. This interpretation is supported by the fact that the adreno-demedullated animals discussed in the present study showed a progressive loss of weight when given 2 per cent NaCl instead of water, whereas in the earlier work they remained in good condition on the salt diet.

The blood sugar curves presented in this paper indicate that the cortical hormones delay the action of insulin in the adreno-demedullated group. They also show that the frequency of cerebral symptoms is not due to the blood sugar level. Assuming that the symptoms occur when the utilization of sugar reaches a certain low value, it appears most probable that the glucose uptake by the brain is higher in the adreno-demedullated group than in the adrenalectomized group at similar blood sugar levels. This suggests that the brain circulation is better in the adreno-demedullated than in the adrenalectomized animals.

Circulatory disturbances in adrenalectomized animals are likewise suggested by the observation that they frequently fail to recover from insulin convulsions on injection of glucose. The absorption of glucose from the peritoneal cavity of these animals is prompt as indicated by sugar measurements. Nevertheless, the rats often remain in a comatose condition. Whether this is due to inadequate brain circulation or whether hypoglycemia induces irreparable brain damage relatively quickly in adrenalectomized animals cannot be decided at the present time. It is, however, more probable that circulatory failure plays the predominant rôle since adrenalectomy predisposes to shock (Swingle, Parkins and Remington, 1941).

SUMMARY

1. The effect of 0.2 u. insulin/kgm. on adreno-demedullated and adrenalectomized rats is similar as far as the minimal blood sugar level is concerned but the rate of fall is more rapid in the adrenalectomized animals.

2. Coma and convulsions occur frequently in the adrenalectomized and rarely in the adreno-demedullated group in spite of similar degrees of hypoglycemia. This difference is seen not only in the gross behavior but also in the E.E.G. which may be normal during hypoglycemia in an adreno-demedullated animal although the same blood sugar level invariably causes coma, disappearance of alpha and appearance of delta potentials in adrenalectomized animals.

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RENAL EXCRETION OF SULFATE[1, 2, 3]

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Received for publication July 3, 1942

For a large number of organic filterable solutes, such as the foreign sugars, the urinary excretion rate is in general proportional to the serum concentration. Under specified conditions the ratio of these two quantities, the clearance, is a constant independent of the serum concentration. Such clearances are closely dependent on the glomerular filtration rate and may indeed for some substances be numerically equal to it. The clearance of each substance is largely independent of the presence or absence of other substances in the urine.

Clearances of ions may be calculated in the same way as those of the organic filterable substances, but their behavior is less simple. In the first place, these clearances are not independent of serum concentration. In the second place, the virtual electroneutrality of the urine means that the sum of the concentrations of cations must always equal that of anions. With each cation an equivalent amount of anion must be excreted, and vice versa. It is manifestly not possible for the excretion of all anions and cations to depend simply on their respective concentrations in serum.

Nevertheless excretion of electrolytes does proceed in an orderly fashion. The experiments to be described attempt to analyze the relationships between electrolyte excretion and certain other variables, in order to formulate the type of general statements which can be made concerning ionic excretion. Sulfate was chosen as a reference substance, partly because of its representative character, partly because its concentration in serum may safely be increased to a greater extent than concentrations of other ions. Excretion of inorganic sulfate following the intravenous injection of various sulfate salts has been compared with the concentration of sulfate in serum, with glomerular filtration, and with the excretion of associated cations and anions.

MATERIALS AND METHODS. Adult female dogs were used in all experiments. An inlying catheter was kept in place, and after a preliminary period of urine collection, a solution of the salt to be studied was injected intravenously. The exact composition and concentration of the solution injected in each experiment is indicated in the tables. The urine specimens during the infusion and during several subsequent collection periods were saved, completeness of collection

[1] A preliminary report of certain of these experiments was presented before the American Physiological Society in 1940. This Journal **129:** P498, (1940).

[2] This paper is based in part upon a thesis submitted by Bernard M. Schwartz to the faculty of the Yale University School of Medicine in candidacy for the degree of Doctor of Medicine, 1939.

[3] Aided by grants from the Ella Sachs Plotz Fund, from the Fluid Research Fund of the Yale School of Medicine, and from the Emerson Fund.

being insured by washing out the bladder with water. The exact time at which each period began or closed was recorded. Samples of blood were obtained from the femoral vein, both before the infusion and at the beginning and end of each urine collection period thereafter. Creatinine, 10 grams in 100 cc. of solution, was injected intraperitoneally just before the salt infusion. Urine and serum samples were analyzed chemically for sulfate, creatinine and various associated cations and anions. Clearances were calculated for each period by dividing the average excretion rate for that period by the mean serum concentration during the period. The latter was determined by the method of Winkler and Parra, which has been previously described (16). Creatinine clearance is assumed to be identical with glomerular filtration rate in the dog (11, 12).

Sodium was determined by the method of Hald (7), sulfate by the method of Cope (1), and chloride by the method of Van Slyke (10). Magnesium and creatinine were determined by methods previously described (14, 16).

Results. In table 1 are presented the results of experiments in which sulfate, alone and in combination with sodium chloride, was injected. In tables 2 and 3 are presented the results of similar experiments in which potassium sulfate and magnesium sulfate, respectively, were employed. The results will be analyzed in several ways.

A. *Relationship between urinary excretion of sulfate and serum concentration of sulfate in all experiments.* In figure 1 the excretion rate of sulfate is plotted against serum concentration in experiments of table 1. Figure 2 is a similar plot showing the results of the experiments of tables 2 and 3. There is evidently considerable variation from experiment to experiment in the exact relationship of excretion rate to serum concentration. Nevertheless, from these figures it appears that there is in general for each experiment a linear relation between the rate of excretion of exogenous sulfate and its concentration in serum. Extrapolations of these lines do not pass through the origin, but intersect the abscissa at various points. The lines of figure 2, representing the excretion of moderate amounts of exogenous sulfate, tend to intersect near the same point, corresponding to some 3 mEq. per liter. Only sodium sulfate could be used to study the excretion of large amounts of sulfate, since the salts of magnesium and of potassium are too toxic. The lines (fig. 1) are steeper than those of figure 2, and when extrapolated cut the abscissa at higher concentrations. It is noteworthy that this linearity held good in spite of the fact that the simultaneous creatinine clearances varied widely (table 1).

B. *Associated excretion of cations.* (1) *Sodium sulfate experiments.* In the four experiments in which sodium sulfate alone was injected, the concentration of sodium in urine at first almost exactly equalled that of sulfate. In subsequent periods the concentration of sodium was usually a little lower than that of sulfate. In experiment 13 of table 1 potassium excretion increased sufficiently to maintain substantial equality in the sums of anions and of cations. The main associate of the sulfate was always sodium, however.

In two of the three experiments in which mixtures of sodium sulfate and sodium chloride were given the quantitative parallelism between urinary sodium

TABLE 1

Intravenous injection of hypertonic solution of sodium sulfate and sodium chloride, singly and in combination

EXPERIMENT NO. DOG WEIGHT, KGM.* SALT GIVEN, mM.**	PERIOD†	DURATION, MINUTES	URINE FLOW, CC. PER MINUTE	CONCENTRATION, m.Eq. PER LITER								CLEARANCE, CC. PER MINUTE			
				Urine					Serum						
				NH_4^+	K^+	Na^+	$SO_4^=$	Cl^-	Na^+	$SO_4^=$	Cl^-	Na^+	$SO_4^=$	Cl^-	Creatinine
									142.4	2.7					
No. 6	1	30	0.23			3	—	—				0.0			
25.0 kgm. A	2	30	15.00			221	—	9							
									177.5	65.3					
Na_2SO_4 206mM.	3	20	12.90			244	244	4				18.0	54		83
									175.5	51.5					
	4	30	7.00			282	309	1				12.0	50		83
									164.5	36.0					
	5	60	2.83			289	365	1				5.1	36		55
									158.0	22.1					
	6	65	1.28			259	363	2				2.1	23		32
									157.4	17.6					
	7	125	1.05			209	293	9				1.4	24		32
									155.3	8.9					
									139.2	2.3	105.9				
No. 7	1	42	0.55			15	16	38				0.0	4	0.2	
24.7 kgm. A	2	39	8.40			188	179	31							
									172.0	29.3	108.4				
Na_2SO_4 103 mM. +	3	33	5.94			229	206	23				8.1	51	1.3	119
									163.5	19.9	110.3				
NaCl 103 mM.	4	68	2.18			244	289	6				3.2	44	0.1	78
									164.2	10.6	110.7				
	5	87	0.86			160	293	4				0.8	31	0.0	57
									165.0	6.5	111.6				
									146.1	2.3	102.7				
No. 8	1	25	0.60			2	28	3				0.0	7	0.0	
24.6 kgm. A	2	48	9.80			215	151	624							
									189.8	38.7	118.9				
Na_2SO_4 154 mM. +	3	54	8.43			276	221	536				12.6	62	3.7	64
									181.0	23.2	123.0				
NaCl 308 mM.	4	125	3.56			255	251	32				5.1	60	0.9	55
									175.6	9.6	125.7				
	5	144	0.92			202	232	9				1.1	36	0.1	53
									177.0	3.7	129.7				
	6	1025	0.75			194	31	132							
									144.4	3.5	113.2				
	7	1725	0.57			149		137							
									144.8		107.4				
No. 9	1	—	—												
25.4 kgm. A	2	45	3.16			173		157							
									167.8		129.0				
NaCl 308 mM.	3	54	1.76			177		150				1.9		2.1	89
									156.2		127.4				
	4	89	1.19			166		155				1.2		1.5	67
									156.1		124.2				
	5	158	1.23			120		138				1.0		1.4	84
									153.0		121.6				
	6	1084	0.54			65		65				0.2		0.3	
									149.2		119.7				
	7	1440	0.79			100		85							

* Letters refer to individual animals.

** The total amount of salt given was dissolved in one liter, except in experiment 13, in which the volume of the infusion was two liters.

† Infusions given during period 2, except as otherwise noted.

TABLE 1—*Concluded*

EXPERIMENT NO. DOG WEIGHT, KGM.* SALT GIVEN† mM.**	PERIOD‡	DURATION, MINUTES	URINE FLOW, CC. PER MINUTE	CONCENTRATION, m.Eq. PER LITER								CLEARANCE, CC. PER MINUTE			
				Urine					Serum						
				NH_4^+	K^+	Na^+	$SO_4^=$	Cl^-	Na^+	$SO_4^=$	Cl^-	Na^+	$SO_4^=$	Cl^-	Creatinine
									141.3	2.2	104.4				
No. 10	1	19	0.11	264		27	46	11				0.6	2	0.0	
19.5 kgm. B	2	38	14.17	0		236	237	24							
									178.2	64.7	90.8				
Na_2SO_4 206 mM.	3	31	7.55	2		297	305	13				13.2	39	1.1	58
									162.3	52.8	96.3				
	4	57	4.32	49		367	391	1				10.0	41	0.0	84
									160.5	30.2	101.3				
	5	121	1.20	13		443	554	3				3.4	39	0.0	60
									149.7	7.8	105.0				
	6	150	0.68	29		407	304	7				1.9	40	0.0	40
									143.7	3.0	107.3				
	7	990	0.20	—		125	90	25							
									142.0	2.0	98.4				
No. 11	1	71	0.11			91	82	191				0.0	2	0.0	
19.3 kgm. B	2	47	14.10			228	238	87							
									184.8	26.4	125.9				
Na_2SO_4 137 mM. +	3	44	7.27			301	110	111				12.0	38	6.4	71
									176.2	15.9	126.3				
NaCl 410 mM.	4	55	2.47			313	170	87				4.4	28	1.6	59
									174.0	14.1	130.7				
	5	139	0.99			267	254	54				1.5	19	0.4	62
									172.6	12.8	137.4				
	6	1174	0.39			287	49	169							
	7	1320	0.29				35	176							
No. 12	1	—	—			119	0	121‡							
									142.5	2.3	92.1§				
24.2 kgm. C	2	44	3.90			143	0	110‡				3.7	0	4.4	75
									138.0	2.3	87.3§				
NaBr 154 mM. before period 1	3	53	8.72			266	278	10‡				—	—	1.1	64
									174.6	51.4	73.5§				
Na_2SO_4 206 mM. during period 3	4	63	5.97			326	338	7‡				12.0	50	0.6	57
									156.2	29.1	83.9§				
									145.0	—					
No. 13	1	24	0.29	—	—	157	58					0.3			
28.0 kgm. D	2	36	5.75	4	34	301	331								
									187.7	90.0					
Na_2SO_4 309 mM.	3	40	13.30	1	35	312	337					23.1	64		126
									171.2	54.8					
	4	63	6.90	1	41	335	401					14.1	70		117
									157.7	28.5					
	5	92	2.96	11	46	348	417					6.7	59		88
									151.7	15.5					
	6	80	1.34	13	62	381	434					3.3	50		92
									152.3	8.6					

‡ Bromide concentrations 9.8, 13.3, 1.5, and 0.5 mM per liter urine respectively in these four periods.
§ Bromide concentrations 16.1, 16.0, 16.1, and 14.5 mM per liter serum respectively in these four samples.

and sulfate is also quite close (expts. 7 and 8 of table 1). Initially the sodium excretion exceeded the sulfate excretion, enough chloride being excreted to make up the difference. In subsequent periods chloride excretion is almost wholly

suppressed, and sodium and sulfate concentrations are nearly equal. In the exceptional experiment (no. 11) chloride excretion continues in considerable amounts during and for some time after the infusion. The sum of chloride and sulfate concentrations first exceeds and then falls somewhat below that of sodium.

(2) *Potassium sulfate experiments.* In the two potassium sulfate experiments the rate of excretion of potassium falls much below that of sulfate. In experiment 15 of table 2 the difference is evidently almost exactly made up by the sodium, the excretion rate of which is considerably above normal.

TABLE 2

Intravenous injection of isotonic potassium sulfate solution

EXPERIMENT NO. DOG WEIGHT, KGM. SALT GIVEN, mM.	PERIOD*	DURATION, MINUTES	URINE FLOW, CC. PER MINUTE	CONCENTRATION, m.Eq. PER LITER						CLEARANCE, CC. PER MINUTE		
				Urine				Serum				
				Na^+	K^+	$SO_4^=$	Cl^-	K^+	$SO_4^=$	K^+	$SO_4^=$	Creatinine
								4.3	2.3			
No. 14	1	32	0.19		42	68	11			2	6	
25.2 kgm.	2	75	2.61		82	219	8					
								7.9	11.7			
K_2SO_4 51 mM.	3	72	0.94		188	269	1			26	28	80
								6.3	7.4			
	4	52	0.40		138	220	5			9	12	46
								6.5	7.0			
	5	144	0.29		145	214	24			6	10	44
								7.4	6.0			
								5.5	2.7			
No. 15	1	21	0.38	90	62	60	74					
16.0 kgm.	2	49	2.08	118	128	242	7					
								8.1	9.4			
K_2SO_4 25.5 mM.	3	59	0.98	125	165	280	3			19	37	76
								8.6	4.6			
	4	89	0.39	75	165	223	6			8	24	70
								6.7	2.9			
	5	45	0.33	58	177	132	6			9	15	63
								6.3	2.9			

* Salt solution injected during period 2.

(3) *Magnesium sulfate experiments.* In the five magnesium sulfate experiments (table 3) there is a close parallelism between sulfate and magnesium in the urine, but the magnesium concentration is always distinctly less than that of sulfate. Chemical analyses for other cations and anions were not done.

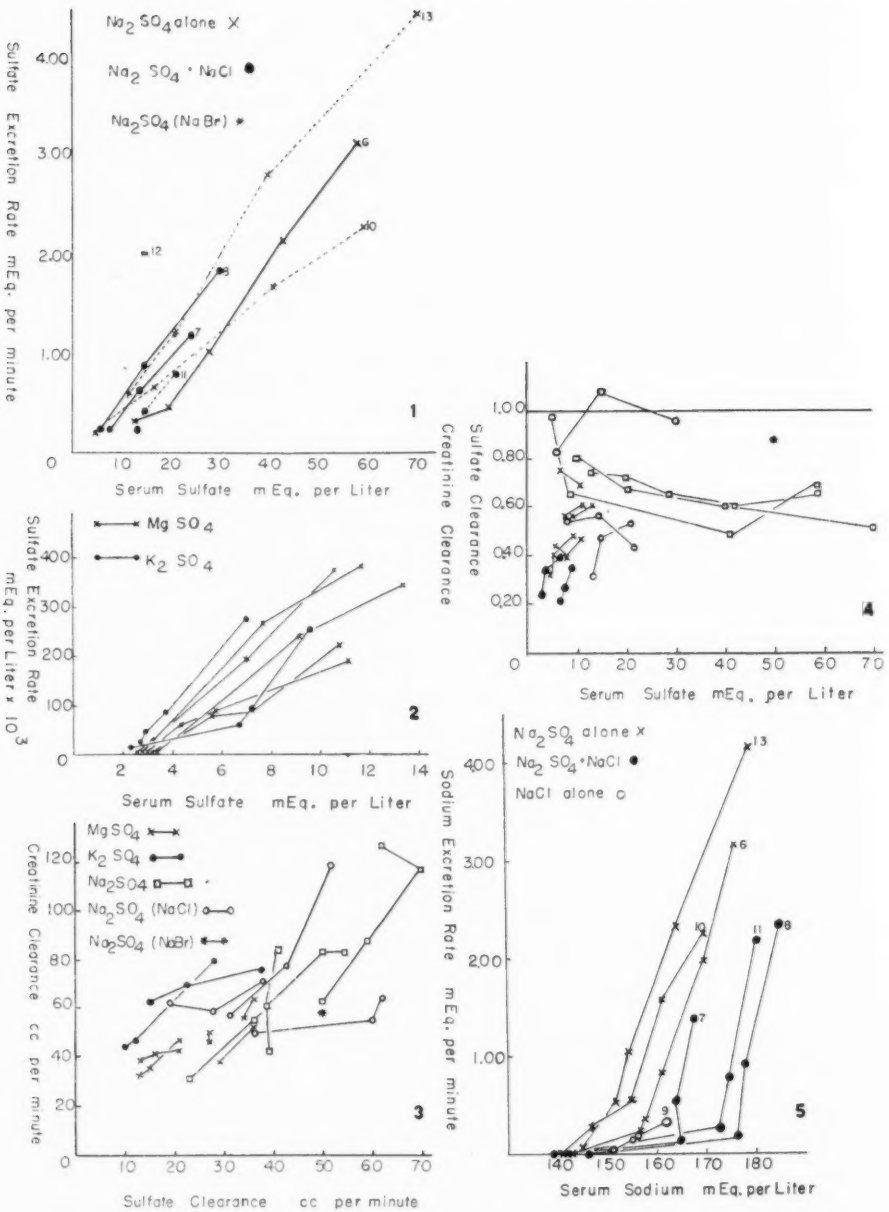
C. *Associated excretion of anions.* (1) *Effects of sulfate on chloride excretion.* The injection of sodium or of potassium sulfate evidently virtually abolished the normal excretion of chloride for some time (table 1, expts. 6 and 10, table 2, expts. 14 and 15). A similar inhibition is however present even when sodium chloride is injected simultaneously with the sodium sulfate (table 1, expts. 7, 8

TABLE 3

Intravenous injection of isotonic magnesium sulfate solution

EXPERIMENT NO. DOG WEIGHT, KGM. SALT GIVEN, mM.	PERIOD*	DURATION, MINUTES	URINE FLOW, CC. PER MINUTE	CONCENTRATION, m.Eq. PER LITER				CLEARANCE, CC. PER MINUTE		
				Urine		Serum				
				Mg^{++}	$SO_4^{=}$	Mg^{++}	$SO_4^{=}$	Mg^{++}	$SO_4^{=}$	Creatinine
						1.9	2.8			
No. 1	1	24	0.42	9	16			2	2	
17.4 kgm.	2	24	3.50	122	171					
						15.2	14.5			
$MgSO_4$ 27.7 mM.	3	33	1.60	168	232			23	34	56
						9.2	9.0			
	4	46	1.50	130	179			24	36	64
						6.8	6.4			
No. 2	1	38	0.16	6	11	1.4	3.4	1	0	
	2	32	0.22	334	464					
						14.2	15.8			
18.8 kgm.	3	40	1.20	199	284			20	27	46
						11.1	10.9			
$MgSO_4$ 24.8 mM.	4	60	1.30	140	190			22	27	49
						6.0	7.5			
	5	1024	0.40	11	57					
						1.2	3.0			
No. 3	1	52	0.29	7	23			2	2	
11.7 kgm.	2	24	1.40	163	217					
						13.7	13.5			
$MgSO_4$ 18.0 mM.	3	71	0.49	307	385			15	21	42
						7.4	6.8			
	4	71	0.27	260	338			12	16	41
						4.9	4.6			
	5	96	0.28	138	185			10	13	39
						3.3	3.7			
						1.8	2.6			
No. 4	1	25	0.40	7	14					
30.3 kgm.	2	35	1.10	211	368					
						13.7	12.7			
$MgSO_4$ 38.5 mM.	3	48	1.40	164	270			20	36	53
						9.6	8.6			
	4	100	0.97	140	206			16	29	38
						7.5	5.5			
						1.4	3.1			
No. 5	1	19	0.11	—	6				0	
18.9 kgm.	2	35	1.70	160	185					
						12.1	13.4			
$MgSO_4$ 25.3 mM.	3	145	0.72	226	311			17	21	46
						8.0	8.3			
	4	68	0.41	193	222			12	13	33
						6.0	6.1			
	5	1085	0.35	192	241			13	15	36
						4.6	5.0			

* Salt solution injected during period 2.


Na2 SO4 alone X
Na2 SO4 + NaCl ●
Na2SO4 (NaBr) *
Sulfate Excretion Rate mEq. per minute
Serum Sulfate mEq. per Liter
1
Mg SO4
K2 SO4
Sulfate Excretion Rate mEq. per Liter x 10^3
Serum Sulfate mEq. per Liter
2
Mg SO4
K2 SO4
Na2SO4
Na2SO4 (NaCl)
Na2SO4 (NaBr)
Creatinine Clearance cc per minute
Sulfate Clearance cc per minute
3
Creatinine Clearance
Sulfate Clearance
Serum Sulfate mEq. per Liter
4
Na2SO4 alone X
Na2 SO4 + NaCl ●
NaCl alone ○
Sodium Excretion Rate mEq. per minute
Serum Sodium mEq. per Liter
5

Figures 1–5

and 11). In these experiments chloride excretion diminishes to virtually nil in spite of a progressive elevation of the chloride concentration in serum and the presence of an excess of sodium chloride in the body requiring excretion. Experiment 9 proves that this inhibition of chloride excretion is related to the simultaneous presence of sulfate, since injection of sodium chloride alone is followed by an increased and approximately parallel excretion of both sodium and of chloride.

(2) *Effects of anions on sulfate excretion.* In figure 1 urinary excretion of sulfate in three separate experiments using the same animal is compared with its serum concentration in three separate experiments (6, 7 and 8 of table 1). (These three experiments are represented by solid lines.) The line furthest to the right (6) represents the excretion after the injection of sodium sulfate alone, the middle line (7) the excretion after injection of a mixture of two parts of sodium sulfate and three parts sodium chloride, while the line furthest to the left (8) represents the excretion after an infusion of a mixture of two parts of sodium sulfate and four parts of sodium chloride. The greater the proportion of sodium chloride given, the greater the excretion rate of sulfate corresponding to any given serum concentration. In other words, increasing the proportion of chloride in the infusion increased the clearance of sulfate. This appears to be a clear effect of an associated anion upon sulfate excretion.

D. *Relation of sulfate excretion to glomerular filtration rate.* In figure 3 creatinine clearance is plotted against simultaneous sulfate clearance for each experiment. A certain general parallelism is evident, but the dispersion of the points is considerable. In figure 4 the ratio of sulfate to simultaneous creatinine clearance is plotted against serum sulfate. No definite relationship is found. This means that the *proportion* of filtered sulfate which is reabsorbed by the tubules is not necessarily greater at high than at low concentrations of sulfate. This is at variance with the findings of Goudsmit, Power and Bollman (6); possible reasons for the differing result will be discussed elsewhere. It has been noted above that in figure 1 the lines relating serum concentration to urinary excretion appear straight, although glomerular filtration varied considerably in each experiment. This suggests that under these particular conditions excretion rate was but little affected by variation in glomerular filtration.

Fig. 1. Relationship between sulfate excretion rate and concentration of sulfate in serum following the injection of sodium sulfate alone and in combination with sodium chloride. Numbers refer to individual experiments of table 1. The solid lines represent three experiments on the same animal, in which differing proportions of sodium chloride to sodium sulfate in the infusion were employed. Other experiments are represented by broken lines.

Fig. 2. Relationship between sulfate excretion and concentration of sulfate in serum following the injection of magnesium sulfate and of potassium sulfate.

Fig. 3. Relationship between sulfate clearance and creatinine clearance.

Fig. 4. Relationship between the ratio of sulfate to creatinine clearance and the concentration of sulfate in serum.

Fig. 5. Relationship between sodium excretion rate and the concentration of sodium in serum. Numbers refer to individual experiments of table 1.

DISCUSSION. The excretion rate of sulfate evidently depends in part upon its serum concentration. This observation is consistent with that of others (6, 8). The straight lines relating serum concentration and excretion rate do not, however, pass through the origin (figs. 1, 2). Algebraically the equation of any such lines is of the form

$$UV = k(S - a), \quad \text{or} \quad k = \frac{UV}{S - a} \tag{1}$$

where UV is the excretion rate, S the serum concentration, k the slope of the line, and a the distance from the origin to the point of intersection of the abscissa. If this equation were to characterize completely the excretion of sulfate, excretion should cease whenever the concentration in serum declines to the value a. Thus a in each experiment would be an apparent "renal threshold" for sulfate excretion. Such is, however, not the case. Even in those experiments in which the value of a approximates that of the endogenous serum sulfate concentration, the equation fails to characterize sulfate excretion properly, since, in point of fact, the excretion of sulfate does continue, though at a much slower rate, at usual endogenous serum concentrations. Indeed sulfate was long ago classified as a "non-threshold" substance since almost no urine is completely free of sulfate (13). Even less adequate is the characterization in those experiments (table 1: 6, 7 and 8) in which the extrapolated lines intersect the abscissa well above the usual endogenous concentration. In fact, a line representing the whole of the experimental data must consist of two parts. The first, valid at elevated serum concentrations, is represented by equation (1). At low concentrations this straight line is replaced by another curve having a much less acute slope. Our data are not adequate to determine whether or not this second portion is exactly a straight line, or whether its junction with the first portion is curved or abrupt. It seems clear, however, that excretion of sulfate is a different function of the serum concentration when it is artificially raised to a high concentration than it is when it lies within the usual range of physiological variation.

Because the lines representing exogenous excretion do not pass through the origin, it is mathematically self-evident that the clearance of sulfate will depend on serum concentration. This may best be seen by calculating the clearance from equation (1):

$$\text{Clearance} = \frac{UV}{S} = k\left(1 - \frac{a}{S}\right) \tag{2}$$

The clearance will evidently be greater the higher the serum concentration. In this respect the clearance of sulfate differs entirely from those of the organic filterable group, whose representative lines do pass through the origin and whose clearances are therefore independent of serum concentration. In those instances in which the value of a corresponds to the usual endogenous concentration of sulfate, the excretion of sulfate is simply proportional to the *increase* in concentration of sulfate in serum (equation (1)). Unfortunately this simple generalization does not hold under all circumstances.

Whole salts rather than individual ions are excreted, since total anion and total cation concentration are always nearly equal. As a necessary consequence of this interdependence of anion and cation the renal excretion rates of both cannot be dependent solely on their respective concentrations in serum. Experiments 7 and 8 of table 1, in which a mixture of sodium sulfate and of sodium chloride was injected, furnish an excellent example of this principle. In the first two periods after injection equal amounts of sodium and of sulfate were excreted in the urine; yet during these same periods the serum concentration of sulfate had varied several fold while that of sodium had changed by only a fraction of its total value. Excretion of sulfate corresponds fairly closely to its concentration in serum, while at the same time the excretion of sodium is dissociated from its serum concentration. Its real determinant appears to be the concentration of sulfate in the urine, enough sodium being excreted to neutralize the sulfate, more or less without regard to its concentration in serum. At least this is the way that the relationship appears. Possibly, if sodium of serum could be increased as much proportionally as is sulfate, this apparent dependence of sodium on sulfate excretion might disappear. This possibility is unfortunately incapable of experimental verification.

There is in fact a linear relationship between excretion rate and serum concentration for sodium as well as for sulfate in these experiments. This is shown in figure 5. The lines following injections of mixtures of sodium chloride and sodium sulfate at first have a very steep slope, then break sharply while the serum concentration is still much elevated. Excretion rate of sodium is thereafter much reduced. Reference to the protocols (table 1) shows the meaning of this. Sodium is excreted very rapidly as sodium sulfate until nearly all the injected sulfate has been eliminated, and then is excreted much more slowly as sodium chloride until the added chloride has been eliminated. In other words, sodium is excreted by different laws depending on the associated anion. A passive rôle of sodium, at least under these circumstances, is again suggested. Although excretion of sodium is functionally related to its concentration in serum, a calculation of sodium clearances alone in these experiments would not clarify the real physiological situation.

The "extra" excretion of sodium after potassium sulfate infusion presents a somewhat more complex problem. This excessive excretion of sodium occurs after ingestion or injection of all potassium salts. Indeed advantage has been taken of this effect in the use of potassium salts as diuretics (9). It is possible that both potassium and sulfate are individually excreted, each according to its own law. Since this would result in an inequality between cation and anion concentration in urine, enough sodium is excreted to preserve electroneutrality. Certainly it is true that, following the injection of various potassium salts, potassium excretion, like sulfate excretion, is closely related to serum potassium concentration and is relatively little affected by the associated anion.

It is not clear whether the increased excretion of potassium after hypertonic sodium sulfate infusions (table 1, expt. 13) represents a similar sort of response, since we have failed to discover any characteristic law of sodium excretion which

would prevent an excretion of sufficient sodium to balance all the sulfate. This particular effect seems to be one expression of a general type of reaction of the organism to any urgent need for water in the excretion of salts. For example, increased excretion of potassium may also be produced by the injection or ingestion of hypertonic sodium chloride solutions (2, 3, 4). A similar excessive renal excretion of potassium may be produced by severe chronic water deprivation, without the injection of any salt (3). On the other hand, little or no increase in potassium excretion is brought about by the injection of isotonic sodium chloride (15). The loss of potassium is frequently associated with the development of hypertonicity in the extracellular fluids, but it is not clear that this is an invariable accompaniment. It may be pointed out that both the development of extracellular hypertonicity and the loss of potassium have one effect in common. They both release intracellular fluid for extracellular distribution. The final effect of both reactions is to diminish the degree to which extracellular fluid contracts. A certain protection to the circulation is thereby provided. Loss of potassium may, therefore, be not wholly without value to the organism.

In the magnesium sulfate experiments both magnesium and sulfate, though originally provided in equimolecular quantities, and distributing themselves through the same compartment of body fluid, obey their separate laws of excretion consistently in all five experiments. The characteristic clearances of magnesium are always regularly a little below those of sulfate. Presumably the excretion of a small amount of some other cation was stimulated, in order to ensure continuous electroneutrality of urine. Unfortunately no analytical data bearing on this point are available.

The presence of sulfate greatly modifies the excretion of chloride, while the effect of chloride on sulfate excretion is not quantitatively so great. Indeed the latter is only important in that it establishes the principle that the characteristic mode of excretion of sulfate is modified by other associated anions. The inhibition of sodium chloride excretion by the simultaneous excretion of sodium sulfate is a much more striking phenomenon, since the usual relation between serum concentration and urinary excretion is exactly reversed. A great excess of sodium chloride awaits excretion and the serum chloride concentration is already elevated; yet the excretion rate of chloride progressively declines and the concentration of chloride in serum rises still further. To use an older terminology, chloride slightly decreases the "renal threshold" for sulfate, while sulfate greatly increases the "threshold" for chloride. This is purely a temporary phenomenon. As soon as the sulfate has been eliminated the sodium chloride is excreted in its usual fashion. In effect, the native salt, sodium chloride, is retained until the foreign salt, sodium sulfate, is preferentially almost completely eliminated. This emphasizes in yet another way the peculiar status of both sodium and of chloride. Changes in the serum concentrations of both appear to be the results rather than the causes of the changes in their respective urinary excretions. In these experiments excretion of each of these ions is throughout exactly conditioned to permit

the elimination of the sulfate as rapidly as possible, without regard to their concentrations in serum.

The discrepancy between our results and those of Goudsmit (6) has already been mentioned. He found that at high concentrations of sulfate in serum the sulfate clearance became nearly equal to the creatinine clearance. We, on the other hand, found at similar high serum levels a very irregular relation between creatinine clearance and sulfate clearance (fig. 4). Sometimes they were nearly equal, sometimes not. No consistent tendency to approach one another with rising concentration, such as Goudsmit found, was present. His experiments meant that the rate of tubular reabsorption of sulfate did not keep pace with the greater amount filtered through the glomeruli at high concentrations. In many of our experiments, on the other hand, the rate of reabsorption increased *pari passu* with glomerular filtration. Nowhere was there any evidence of a maximal rate of reabsorption of sulfate, such as was suggested by his experiments. A possible explanation may be found in the way the two groups of experiments were conducted. Goudsmit regularly gave his animals a large amount of saline by stomach tube an hour or so before the experiment, in order to provoke a profuse diuresis. We have already seen in our experiments that administration of sodium chloride along with the sulfate increases the clearance of sulfate and diminishes its tubular reabsorption. It seems entirely likely that a similar effect of sodium chloride was active in Goudsmit's experiments. Certainly in the single experiment of ours (table 1, expt. 12) in which sodium bromide was injected prior to the experiment, the subsequent sulfate clearance closely approached that of creatinine.

The very rough relationship between creatinine and sulfate clearances (fig. 3) indicates that glomerular filtration is only one of several determinants of sulfate clearance. Of course these were all normal animals; were subjects with impaired glomerular filtration included, the relationship might well have been more striking. It has been noted that many of the lines representing sulfate excretion are straight, in spite of markedly varying glomerular filtration. This would seem to mean that, irrespective of the amount delivered to them in the glomerular filtrate, the tubules leave in the urine an amount proportional to the increase in serum concentration ($S - a$). This relationship may of course well be fortuitous, due to the particular way in which these experiments were conducted.

There is much evidence that the kidney is in some way limited in its ability to produce a hypertonic urine (5, 13). Nothing in these experiments, however, suggests that this apparent osmotic limitation in any way conditioned the excretion of ions. Only moderately hypertonic solutions were used, so that considerable water was always available for excretion. Enough water was taken from the infusion itself and from the body store to keep the concentration of salts in the urine well within the normal range. The highest total osmolar concentration in urine did not usually coincide with the maximal excretion rate of salt immediately after infusion, but appeared later, after the excretion had declined more nearly toward normal. This seems good evidence against total

osmotic limitation of excretion, since if this were the limiting factor one would expect the urines of several periods after infusion to have the same "maximal" total osmolar concentration.

SUMMARY AND CONCLUSIONS

1. The excretion of sulfate and of other anions and cations following intravenous infusions of various sulfates in the dog has been studied.

2. Injected sulfate is excreted according to a different law than endogenous sulfate.

3. At high concentrations of sulfate in serum, sulfate excretion is in part a function of serum concentration, and at times is nearly proportional to the increase in concentration.

4. Sulfate clearance varies systematically with serum concentration, increasing as the serum concentration rises.

5. Sulfate excretion is in the main independent of the associated cation.

6. Sulfate excretion is somewhat increased by the simultaneous injection of sodium chloride.

7. Chloride excretion is almost completely repressed by sulfate excretion, even when a large sodium chloride infusion is given simultaneously with the sulfate infusion.

8. The extent to which ions may be excreted according to their own characteristic laws and the means by which electroneutrality of urine is maintained are discussed in the light of these experiments.

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THE RÔLE OF THE ANTERIOR PITUITARY IN THE MAINTENANCE OF NORMAL BLOOD SUGAR LEVELS AND IN THE PHYSIOLOGICAL MOBILIZATION OF LIVER GLYCOGEN[1]

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Received for publication July 6, 1942

In an earlier paper it has been shown that the intravenous infusion of adrenaline produces only a very slight rise in blood sugar in hypophysectomized dogs in contrast to the marked hyperglycemia it produces in normals. This phenomenon cannot be explained on the basis of inadequate liver glycogen stores, since some of our hypophysectomized dogs had liver glycogen stores within normal limits, others had smaller stores, but all had amounts of liver glycogen which, if present in normal dogs, would have been sufficient to have produced a marked hyperglycemia (de Bodo, Bloch and Gross, 1942). On the basis of these earlier experiments it was concluded that in the absence of the anterior pituitary there is an impairment in the mobilization of liver glycogen by infused adrenaline.

With this conclusion in mind the question arose as to whether the liver glycogen of hypophysectomized dogs is also resistant 1, to adrenaline secreted under physiological conditions, and 2, to other glycogenolytic agents which may function under physiological conditions. If mobilization in response to these agents is also impaired by hypophysectomy, then low post-absorptive blood sugar values should be found coexisting with adequate liver glycogen stores in hypophysectomized dogs.

The literature dealing with the post-absorptive blood sugar levels of hypophysectomized animals is contradictory. Some investigators have reported values lower than those found in normal animals, others have found no change in post-absorptive blood sugar levels after hypophysectomy. This literature is thoroughly reviewed by Houssay (1935) and by Van Dyke (1936, 1939). All investigators are agreed that hypophysectomized animals develop hypoglycemia after fasting, and have a tendency to go spontaneously from an apparently normal state into hypoglycemic shock. However, all these workers have made only random observations of the post-absorptive blood sugar levels of hypophysectomized animals, but have not followed them daily from the time of the hypophysectomy. In view of these facts we have made a systematic study of the effect of hypophysectomy on the post-absorptive blood sugar levels and on the liver glycogen contents of a series of dogs.

METHODS. Dogs were used exclusively in this study. Hypophysectomy was performed by the oral approach (except when otherwise specified) and the pituitary gland removed in one piece, which included the pars distalis, pars

[1] This study was aided by a grant (to R. C. de Bodo) from the American Philosophical Society.

intermedia, pars nervosa, and pars tuberalis. (For further details see de Bodo et al., 1942.) The animals were maintained on a constant diet for two weeks prior to hypophysectomy and throughout the period of observation following hypophysectomy. In every case it was ascertained that the allotted ration was consumed and retained.

Post-absorptive blood sugar levels (17 hrs. after the last feeding) were determined prior to hypophysectomy and almost daily thereafter (except when otherwise specified). In addition, in certain instances blood sugar samples were drawn 24 hours after the last feeding. Immediately prior to the termination of each experiment by the sacrifice of the animal, liver samples for glycogen determination were excised under local anesthesia. The blood samples were drawn without the use of any anesthesia. Blood sugar determinations were made by the Hagedorn-Jensen method (1923), using the Somogyi (1930) acid-zinc filtrate. Liver glycogen determinations were made by a modified Pflüger method (Bodo and Neuwirth, 1933).

At autopsy the organs were fixed in formalin and after 24 hours in the fixative the weights of the endocrine glands were determined, with special attention to the adrenals, pancreases, and gonads. Histological studies were made of sections of a block including the body of the sphenoid bone, the fibrous tissue occupying the sella turcica, and the overlying brain tissue in each animal. In addition histological studies were made of the organs of each animal, with special attention to the thyroids, adrenals, gonads, and pancreas, as well as to the removed pituitary gland. These histological studies were made by Dr. David Marine, Director of the Laboratories of Montefiore Hospital, New York City.[2]

RESULTS. In figures 1 to 7 we have presented graphically the day-to-day variations observed in the post-absorptive blood sugar levels of seven hypophy-

Figs. 1–7. Blood sugar changes in 7 dogs during the period following hypophysectomy, with liver glycogen values found at the termination of each experiment. The numbers on the abscissae represent days after hypophysectomy. The numbers on the ordinates to the left of each curve represent blood sugar in milligrams per cent. The numbers on the ordinates to the right of each curve represent the liver glycogen in per cent. The encircled dots represent post-absorptive (17 hrs. after last meal) blood sugar values before hypophysectomy. The simple dots represent post-absorptive (17 hrs. after last meal) blood sugar values after hypophysectomy. The solid triangles represent blood sugar values 24 hours after last meal. The solid vertical columns represent liver glycogen values. The hatched areas represent range of post-absorptive (17 hrs. after last meal) blood sugar concentration of 18 normal dogs maintained for a period of weeks on the same diet as the hypophysectomized dogs.

Fig. 8. Fluctuations in post-absorptive blood sugar values of 6 normal dogs during a period of three weeks. The numbers on the abscissa represent days of the experiment. The numbers on the ordinate represent blood sugar in milligrams per cent. Each of the six symbols used in this figure represents the post-absorptive (17 hrs. after last meal) blood sugar values of one normal dog. Note that the fluctuations in the values for each dog do not exceed 10 mgm. per cent.

[2] A detailed report of the histological studies is now in preparation and will be published in the near future, together with an analysis of the changes in weight of the endocrine organs and their significance.

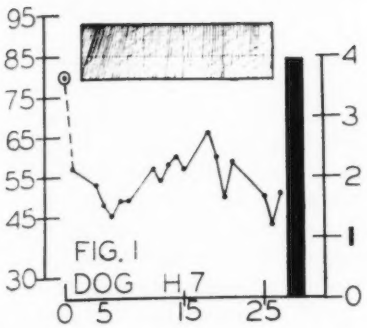
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FIG. 1
DOG H 7

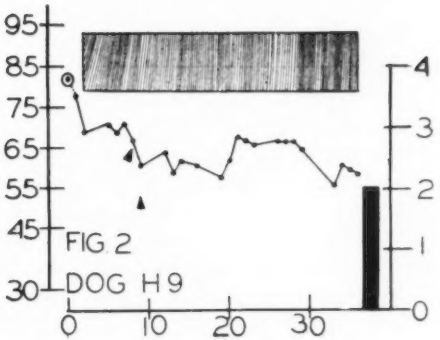
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FIG. 2
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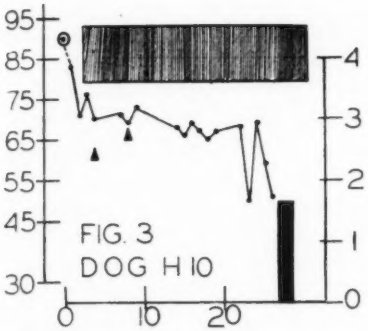
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FIG. 3
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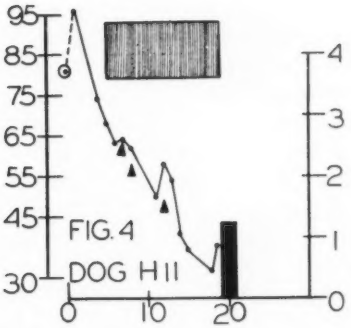
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FIG. 4
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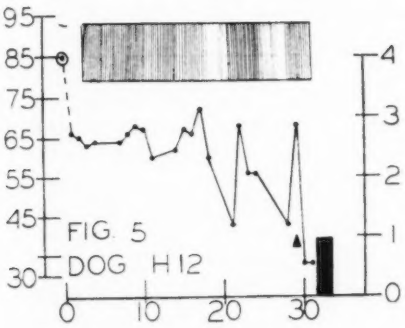
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FIG. 5
DOG H 12

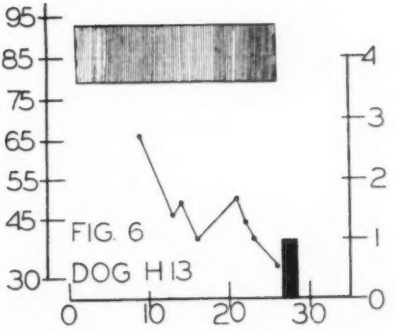
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FIG. 6
DOG H 13

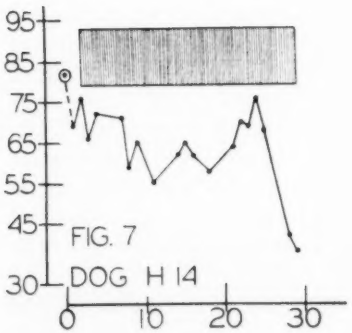
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FIG. 7
DOG H 14

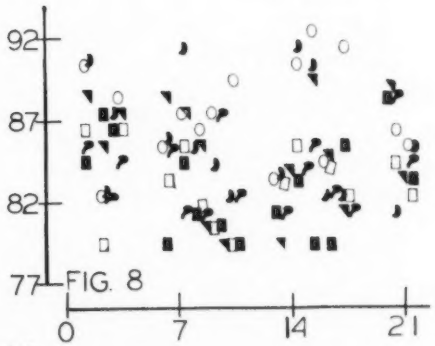
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FIG. 8

Figs. 1–8

sectomized dogs. It can be seen that in the first two post-operative days a sharp decrease in the post-absorptive blood sugar value is apparent, except in the case of dog H11. This dog was the only one of those observed which had a higher post-absorptive blood sugar concentration the day after hypophysectomy. However, it should be noted that shortly thereafter the blood sugar concentration fell to below its preoperative level and continued to fall from then on.

During the weeks of observation following the hypophysectomy the post-absorptive blood sugar concentrations of each of these seven dogs fluctuated from day to day but were always at levels substantially lower than those maintained before hypophysectomy. The hatched area in each figure represents the range of post-absorptive blood sugar concentration (79–93 mgm. per cent) of 18 normal dogs maintained for a period of weeks on the same constant diet as that fed to the hypophysectomized dogs.

A glance at the seven curves (figs. 1–7) reveals the striking range of the fluctuations in post-absorptive blood sugar values in the hypophysectomized dogs during the period of observation. In figure 8 the post-absorptive blood sugar concentrations of 6 normal dogs studied for a period of three weeks are given. As can be seen the fluctuations in the post-absorptive blood sugar values of each individual dog did not exceed 10 mgm. per cent, whereas among the hypophysectomized dogs the most limited range seen was that of dog H9 (56–71 mgm. per cent = 15 mgm. per cent, even disregarding the blood sugar value of 78 mgm. per cent on the first post-operative day). In the other hypophysectomized dogs the ranges in milligrams per cent were 26, 23, 39, 38, 42 and 33.

The experiments on dogs H11, H12, and H13 were terminated when their blood sugar concentrations fell to 33 to 38 mgm. per cent, at which time these dogs refused to eat voluntarily and vomited the food fed by stomach tube. With blood sugar values of 32 to 33 mgm. per cent H11 was unable to walk, H12 showed twitches, and H13 was ataxic. None of these dogs showed actual convulsions or coma. No attempt was made to raise the blood sugar level of these dogs either by sugar infusion or by the administration of adrenal cortical extract. Liver samples were taken, under local anesthesia, for glycogen determinations, and the animals were then sacrificed. The experiment on dog H14 was terminated by the death of the animal. The blood sugar concentration fell to 38 mgm. per cent, the animal was ataxic, vomited the food fed by stomach tube, and was found dead the following morning. Thus, due to the autolysis that set in, it was impossible to obtain valid liver glycogen values or to make adequate histological studies of the sella turcica or organs of this animal.

The experiments on dogs H7, H9 and H10 were terminated while their post-absorptive blood sugar concentrations, although markedly lower than those found in normal animals, were still considerably higher than those found in dogs H11, H12, H13, and H14 at the time they were sacrificed. The symptoms observed in these latter dogs when their blood sugar concentrations fell to 33 to 38 mgm. per cent were never seen in dogs H7, H9, and H10.

Dog H11 showed the most abrupt and steady fall in post-absorptive blood

sugar level in the weeks following hypophysectomy. Paradoxically, this is the only animal of those presented here which had in one section of the sella turcica some blurred cells which might have been anterior pituitary cells. Also the interstitial cells of the gonads of this dog showed some signs of activity, and the adrenal cortices were not as atrophic as those of some of the other animals. In all the other dogs histological examination established the complete absence of all pituitary tissue.

All these dogs had very small adrenal glands which showed the cortical atrophy characteristic of total hypophysectomy (de Bodo and Marine), although the degree to which the atrophic changes had progressed varied somewhat from animal to animal. No correlation could be detected between the rapidity and constancy with which the post-absorptive blood sugar level fell and the degree of adrenal cortical atrophy. For example, dog H10 showed a much greater atrophy than did dog H13 or dog H11, although its blood sugar concentration never fell as low.

TABLE 1

Simultaneously determined blood sugar and liver glycogen values of hypophysectomized dogs

DOG NUMBER	BLOOD SUGAR*	LIVER GLYCOGEN	DOG NUMBER	BLOOD SUGAR*	LIVER GLYCOGEN
	mgm.%	*G.%*		*mgm.%*	*G.%*
H5	54	4.42	H16	34	2.04
H6	54	4.02	H10	51	1.65
H7	52	3.95	H11	38	1.22
H8	53	2.86	H12	33	0.94
H15	45	2.86	H13	33	0.98
H9	59	2.05			

* Postabsorptive value.

Of the four dogs which showed the extremely low blood sugar concentrations of 33 to 38 mgm. per cent, three (H12, H13, H14) had been given insulin (0.025 U/kilo intravenously) on two previous occasions. Dog H11 had never been given insulin. The other three of the hypophysectomized dogs studied (H7, H9, H10) were never given insulin. On the basis of this and many other experiments on hypophysectomized dogs not included here, we believe that insulin administration may be one factor which increases the tendency to "spontaneous" hypoglycemic crises in the hypophysectomized animal, even though the animal recovers from the immediate effects of the drug after the intravenous administration of sugar. In other words, though the dog may be brought out of the insulin coma by intravenous glucose and may then appear well, resume activity and eat voluntarily, still a few days after insulin the animal shows a more pronounced tendency to hypoglycemic crises than do hypophysectomized dogs which have never been given insulin.

The amounts of glycogen contained in the livers of these hypophysectomized dogs at the time when the last blood sample was drawn for glucose determination are shown in figures 1 to 6 and in table 1. We have also included in table 1

the results obtained on 5 dogs, hypophysectomized by the temporal approach, whose blood sugar values were not determined daily. Their blood sugar was determined on several occasions, including of course a determination made immediately prior to the excision of the liver samples for glycogen determination. These animals were not 100 per cent total hypophysectomies, in that the histological examination revealed small remnants of anterior pituitary tissue in their sellae turcicae. However, since these hypophyseal remnants were not functionally adequate to prevent the atrophic changes in the adrenal cortices, thyroids, and gonads characteristic of our totally hypophysectomized animals, and since these dogs reacted to insulin and to fasting exactly as did the histologically 100 per cent hypophysectomized animals, we regard these dogs as functionally hypophysectomized.

Considering the figures in table 1 it can be seen that in hypophysectomized dogs a liver glycogen concentration of approximately 4 per cent can coexist with a post-absorptive blood sugar of 52 to 54 mgm. per cent (dogs H5, H6, H7). This amount of liver glycogen is within the normal range, but in a normal dog it would never coexist with such a low blood sugar concentration. Normal dogs with liver glycogen stores in this range have blood sugar values of 79 to 93 mgm. per cent (average 84 mgm. per cent) (de Bodo et al., 1942), about 30 mgm. per cent greater than those found in these hypophysectomized dogs. Not every hypophysectomized dog with post-absorptive blood sugar values of approximately 50 mgm. per cent had as much as 4 per cent liver glycogen. As can be seen in table 1, some (dogs H8, H15, H9, H10) had less liver glycogen.

In dogs H11, H12, and H13 the extremely low blood sugar concentration of 33 to 38 mgm. per cent coexisted with a liver glycogen concentration of approximately 1 per cent. Liver glycogen values of this order may be found in normal dogs only after a period of 8 to 10 days of fasting, but when found they coexist with blood sugar concentrations of 58 to 76 mgm. per cent (average 65 mgm. per cent) (de Bodo et al., 1942), again about 30 mgm. per cent greater than that found in dogs H11, H12, and H13. In dog H16 a blood sugar value of 34 mgm. per cent coexisted with a liver glycogen content of 2.04 per cent. This relatively large amount of liver glycogen with such an extremely low post-absorptive blood sugar value was an unusual finding in our series. In short, the liver glycogen values found in the hypophysectomized dogs listed in table 1 vary widely, but it should be noted that in every instance the coexisting blood sugar concentrations were markedly lower in these animals than they would have been in normal animals with the corresponding liver glycogen values.

In addition to the determination of the daily post-absorptive blood sugar values, the 24 hour fasting blood sugar level was also determined in dogs H9, H10, H11 and H12. These results are shown in figures 2, 3, 4 and 5. It can be noted that, as would be expected, these values are all lower than the corresponding post-absorptive values. However, of all the figures obtained only two (dog H11, 12 days post-operatively and dog H12, 29 days post-operatively) were lower than 50 mgm. per cent. Soskin et al. (1935–6, 1938, 1939) used the 24 hour fasting blood sugar level as a criterion for the completeness of their

hypophysectomies, accepting as completely hypophysectomized only those animals with blood sugars below 50 mgm. per cent. If we were to accept this we would have to regard as incompletely hypophysectomized dogs H9 and H10, each of which had on two occasions 24 hour fasting values over 50 mgm. per cent (65, 53; 63, 68 mgm. per cent), and which were subsequently proved by histological study to be 100 per cent completely hypophysectomized.

CONCLUSIONS AND DISCUSSION. From the results presented here it is apparent that within two days following hypophysectomy the post-absorptive blood sugar of dogs falls to a level considerably below its pre-operative value to which it never returns. During the weeks subsequent to hypophysectomy the post-absorptive blood sugar fluctuates widely around the lower level with a tendency to fall further rather than to rise. The blood sugar never falls from normal to hypoglycemic shock levels without passing gradually (with or without marked fluctuations) through successively lower stages. Day-to-day observation of the post-absorptive blood sugar level of hypophysectomized dogs makes it possible to detect impending hypoglycemic crisis.

Since the animals on which these observations were made were found histologically to be 100 per cent completely hypophysectomized and since all had the adrenal cortical atrophy which is as characteristic of the hypophysectomized dog (de Bodo and Marine) as it is of the hypophysectomized rat (Smith, 1930), we believe that the inability to maintain normal blood sugar levels is also a characteristic of complete hypophysectomy.

Houssay (1935) does not consider subnormal blçod sugar values characteristic of hypophysectomy in the dog. In one of their papers Houssay and Biasotti (1931a) reported blood sugar levels below normal in only 2 out of 5 of their hypophysectomized dogs. However, it should be noted in this connection that Houssay (1936) has stated that adrenal cortical atrophy occurs "less frequently" in hypophysectomized dogs than in hypophysectomized rats. In a paper in which Houssay and Biasotti (1931b) presented histological data, only 1 of the 5 hypophysectomized animals studied showed marked adrenal cortical atrophy, 1 showed moderate atrophy, and the remaining 3 had normal adrenal cortices. In a later paper Houssay (1933) charted the weights of the adrenal glands of 20 of his hypophysectomized dogs, on which no blood sugar findings were presented. We have related the weights of the adrenal glands as given in Houssay's chart to the body weights of his animals. This reveals that only 7 out of his 20 hypophysectomized animals showed a degree of adrenal atrophy comparable to that which we (de Bodo and Marine) observed in all our 100 per cent completely hypophysectomized animals.

Chaikoff et al. (1935) found low post-absorptive blood sugar values in only 2 out of 7 hypophysectomized animals studied. Unfortunately no statements are made as to the condition of the adrenal cortices of these animals.

On the other hand, our results are in agreement with those of Smith et al. (1936) who, although they did not follow blood sugar levels daily before and after hypophysectomy, found that the post-absorptive blood sugar level of hypophysectomized monkeys (33 observations on 9 animals) varies around an

average value of 59 mgm. per cent as contrasted with an average of 110 mgm. per cent found in normal monkeys (53 observations on 24 animals).

The characteristically low post-absorptive blood sugar level of hypophysectomized dogs is found in the presence of fairly large amounts of liver glycogen. The co-existence of these two findings requires further comment. The blood sugar level is determined by the ratio of glucose production to glucose utilization. There is no general agreement as to the effects of hypophysectomy on glucose utilization. Whereas Chambers et al. (1935) were unable to detect any change, and Soskin et al. (1938, 1939) found it decreased, Fischer, Russell and Cori (1936) and Russell (1942a, 1942b) found increased glucose utilization after hypophysectomy in the rat, as did Greeley (1940) in the rabbit.

Assuming that the latter view is correct and that glucose utilization is increased after hypophysectomy, this fact alone could not account for the low blood sugar levels found in our hypophysectomized animals in the presence of the amounts of glycogen contained in their livers. Russell (1942a) claimed that glucose utilization is *twice* as great after hypophysectomy in the rat. As has been shown by Bodo, Barker and Benaglia (1938), normal dogs in the post-absorptive state maintained normal blood sugar levels while performing moderate muscular exercise, during which their glucose utilization was proved to be *four times* increased as determined by the respiratory metabolism. It is clear then that the production of glucose in the normal animal is capable of keeping pace with even a fourfold increase in utilization.

Therefore it is obvious that the low post-absorptive blood sugar values in our hypophysectomized animals cannot be accounted for *solely* on the basis of increased utilization. There must be decreased production of sugar. There is ample evidence that the production of sugar from non-carbohydrate sources is deficient after hypophysectomy, but since our animals had liver glycogen reserves (in some cases within normal limits) the defective formation of sugar from non-carbohydrate sources cannot have been the *only* limitation on glucose production. We must therefore conclude that there is *also* a defective glucose formation from liver glycogen. In other words, after hypophysectomy there is an impairment in the physiological mobilization of liver glycogen.

There is convincing evidence available that when the blood sugar of a normal animal is markedly lowered (to a "critical level") by large doses of insulin, adrenaline is secreted—as demonstrated on the animal's sensitized denervated heart—which in turn mobilizes the liver glycogen (Cannon et al., 1924). It is questionable whether adrenaline secretion occurs when the blood sugar is not lowered as drastically but only to such a slight degree as occurs under truly physiological conditions, as in the course of moderate muscular exercise. In the paper referred to above (Bodo et al., 1938) it was demonstrated that during moderate muscular exercise such liver glycogen mobilization as is necessary to maintain normal blood sugar levels occurs not only in normal but also in adrenal-inactivated (right adrenal removed, left denervated and demedullated) and liver-denervated animals. Therefore liver glycogen mobilization can be effected in non-hypophysectomized dogs by agents other than adrenaline and liver nerves.

In our hypophysectomized animals the blood sugar levels were so low that it is probable that not only were these agents called out but also adrenaline must have been secreted. Cope and Marks (1934–1935) have shown that hypophysectomy does not prevent the secretion of adrenaline in response to insulin hypoglycemia. Our own histological studies have shown that the adrenal medullae after hypophysectomy do not become atrophic but rather hypertrophic. The cells of the medullae are large, columnar, suggestive of great secretory activity (de Bodo and Marine). Since in these hypophysectomized dogs of ours the low blood sugar values coexisted with adequate liver glycogen stores it is apparent that neither secreted adrenaline nor the other physiological agents which are capable of mobilizing liver glycogen in the non-hypophysectomized animal are effective in the absence of the anterior pituitary. This impaired mobilization of liver glycogen must be one of the factors responsible for the characteristically low post-absorptive blood sugar level of hypophysectomized dogs.

SUMMARY

1. Within two days following hypophysectomy the post-absorptive blood sugar of dogs falls to a level considerably below its pre-operative value to which it never returns.

2. During the weeks subsequent to hypophysectomy the post-absorptive blood sugar fluctuates widely around the lower level with a tendency to fall further rather than to rise.

3. With these markedly low post-absorptive blood sugar levels some of the hypophysectomized dogs had liver glycogen stores within normal limits, others had smaller stores, but all had amounts of liver glycogen which, if present in non-hypophysectomized dogs, would have been sufficient to have maintained normal blood sugar levels.

4. In the absence of the anterior pituitary there is an impairment in the mobilization of liver glycogen by secreted adrenaline and also by the other physiological agents which are capable of mobilizing it in the non-hypophysectomized animal.

5. In the absence of the anterior pituitary there is an inability to maintain normal blood sugar levels.

6. The impairment in the physiological mobilization of liver glycogen is one of the factors responsible for the characteristically low post-absorptive blood sugar level of hypophysectomized dogs.

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THE CONTROL OF CLONIC RESPONSES OF THE CEREBRAL CORTEX

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Received for publication July 7, 1942

In a previous communication (Rosenblueth and Cannon, 1942) a study was made of some features of the tonic-clonic, selfsustained responses of the cerebral cortex to electric stimulation. The responses were found similar in different cortical areas. When activity spread to several areas a physiological coupling was revealed by the temporal correlation of their clonic bursts and usually by the simultaneity of the sudden end of the response at all the regions involved.

The present study attempts an analysis of the mechanism by which different cortical regions may become coupled during clonic activity, and also an elucidation of the factors which determine the rate of clonus, and the reason for the simultaneous abrupt end of the responses in several areas.

METHOD. Young Rhesus monkeys were used, anesthetized with chloralose (0.06 to 0.1 gram per kgm., intravenously). The left cerebral hemisphere was largely exposed, and the right cranium also was opened to permit the approach to the arm region of the motor and sensory areas on that side.

Two light brass stands were firmly screwed into the remaining bone. To these stands were attached 6 pairs of silver electrodes which were placed on various cortical areas and were used for either stimulating or recording. Even marked movements of the head of the animals did not disturb the contact of the electrodes with the brain.

Repetitive stimuli capable of producing tonic-clonic responses were obtained from a Harvard induction coil with a 1.5 v. cell in the primary. The stimulating electrodes were applied by hand. The single-shock stimuli were condenser discharges with a time constant of approximately 0.8 msec. and with a strength of 5 to 30 v. Frequencies of 0.5 to 4 per sec. were used, regulated by changing the bias of a thyratron with a potentiometer. The single shocks were delivered through one of the fixed pairs of electrodes on the cortex.

Both for stimulation and for recording, the interelectrodal distances were from 2 to 5 mm.

The cortical electric responses were recorded by means of a Grass 6-channel set of ink-writing, moving-coil galvanometers and associated resistance-capacity coupled amplifiers. The input was on push-pull for each of the recording pairs of electrodes. The animal was grounded through a diffuse lead attached to either temporal muscle. The records in these conditions are quite independent.

RESULTS. A. *The control of clonic activity by single shocks applied to the cortex.* Rosenblueth and Cannon (1942) showed that stimulation of the cortex by single shocks in the course or shortly after the end of a tonic-clonic sequence could elicit

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clonic-like responses. This observation led to the expectation that the rate of clonus could be controlled, and clonic activity could be prolonged by means of trains of shocks with appropriate frequencies. The expectation was confirmed, as follows.

Series of clonic discharges were observed in 3 different conditions: *a*, during a selfsustained response which was not modified by additional single-shock stimulation—the term "selfsustained" will be used to denote this clonic activity, emphasized, when convenient, by the adjective "undriven"; *b*, in the course of a selfsustained response the rate of a clonus could be controlled by appropriate low frequency stimuli—these bursts will be referred to as "driven or controlled" selfsustained clonus; *c*, a clonic response could be prolonged beyond its inherent duration by appropriate single-shock stimulation—this clonus will be designated by the term "prolonged." In order to decide whether single shocks were controlling a selfsustained clonic response the frequency of the shocks was usually varied in the course of the observation. When the clonus followed these variable rates a positive correlation was inferred—i.e., a non-fortuitous coincidence. The prolongation of a clonic series was evidenced not only by the duration, but also by the immediate cessation of the responses upon discontinuance of the stimuli.

For single-shock stimulation to be effective in the control and prolongation of a clonic response several conditions had to be satisfied. *a*. The stimuli had to be stronger than a given critical voltage. It is obvious, therefore, that the threshold of certain cortical elements should be attained. *b*. An appropriate area of the cortex had to be stimulated. This condition is detailed in section B. *c*. It was necessary that the rate of the shocks be adequate, as follows.

A shock applied shortly (within about 0.3 sec.) after a spontaneous clonic burst had occurred, failed to activate the cortex. Hence, when single shocks were repeated faster than about 3.5 per sec. the clonic bursts were never seen to follow each stimulus. The elements of the cortex involved in a clonic response behave, therefore, as if they have a prolonged functional refractory period of about 0.3 sec. This imposes an upper limit on the rate of the effective driving shocks.

Although the maximal rate of clonus which could be imposed at any of the areas tested was about 3.5 per sec., this rate was attainable only early during the clonic response to a prolonged, intense, rapid stimulation. If the stimuli which elicited a tonic-clonic response were weak, or were applied for a brief period, or if a tonic-clonic response had already proceeded for some time, then the imposed rate of clonus could usually follow only lower frequencies. Whenever the rate of driving stimulation was higher than the maximal rate to which the cortex could respond at the time, clonic bursts were elicited by alternate shocks. Responses to every 2nd, or even to every 3rd shock were therefore often seen even though the frequency of stimulation was only 3 per sec. or less.

In figure 1A is illustrated a typical 1:2 alternation. All the active areas share alike in this alternation; this was usual. Figure 1B illustrates an exceptional instance of alternation; the following cycles repeat regularly: short latency → long latency → no response.

If in the course of a clonic response single-shocks were applied with a frequency

slower than that of the prevailing intrinsic rhythm, they did not gain control of the rate. Even if one of the shocks elicited a burst, the next clonic complex occurred before the following shock, and there was no correlation between the stimuli and the clonus.

The rate of stimulation, for prolongation of a clonic response, could not be slower than about 0.7 per sec. Intervals greater than 1.5 sec. invariably resulted

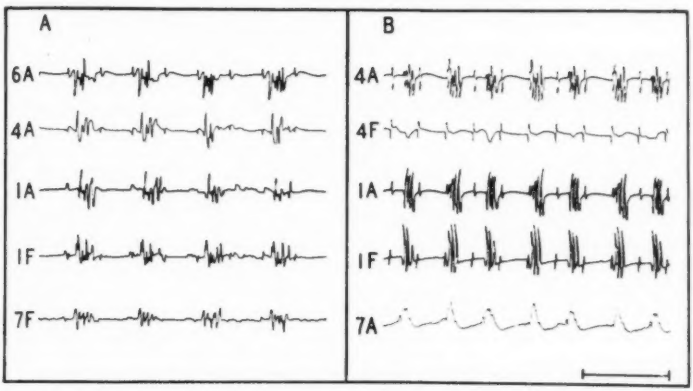

Fig. 1. Alternation of clonic responses to single shocks applied to the cortex. In these and the following figures the records show the electric activity of various cortical areas, unless otherwise stated. The order in which the recording areas are listed corresponds to the successive tracings from above downward. The numbers correspond to Brodmann's (1905) classification. The letters F, A and L, when they follow a number, indicate that the record is from the face, arm or leg division of that area. The letters R or L, when they precede a number, indicate the right or left hemisphere; when there is no preceding letter the area was in the left hemisphere. The speed of the tracings is indicated by the 1-sec. time calibration at the right lower corner. The records all begin some time after a brief (2 to 5 sec.) period of faradic stimulation at tetanic frequency (referred to as rapid stimulation) had initiated a tonic-clonic response in the cortex.

A. Records: *6*A, *4*A, *1*A, *1*F, and 7F. Previous rapid stimulation of *6*A. Single shocks were applied to *8*, as indicated by the artifacts, particularly recognizable in *6*A. The clonic bursts occur in response to every other shock.

B. Records: *4*A, *4*F, *1*A, *1*F, and 7A. Previous rapid stimulation of *4*A. Single shocks were applied to *6*, as indicated by the artifacts in *4*F. The responses follow the stimuli according to the repeated cycle: short latency → long latency → no response. This unusual mode of alternation endured for 30 sec. and was then followed by one of the habitual modes.

in failure of the clonic responses to follow later stimuli. There is thus a lower limiting frequency of stimulation below which clonic bursts cannot be sustained.

It is interesting that the two limiting frequencies mentioned (3.5 and 0.7 per sec.) coincide approximately with the limits of clonic frequency in selfsustained, undriven responses (3 and 1 per sec.; Rosenblueth and Cannon, 1942).

The degree of prolongation of a clonic response by single-shock stimulation depended on the rate of the stimuli. The results were not accurate enough for a precise quantitative statement, but as a rule a frequency of about 3 per sec. pro-

longed the response more than did a rate of 1 per sec. In no instance could the discharges be prolonged indefinitely—i.e., beyond about 5 min. The prolongation depended not only on the rate of the single stimuli but also on the degree of rapid stimulation applied to originate the tonic-clonic response. The same fre-

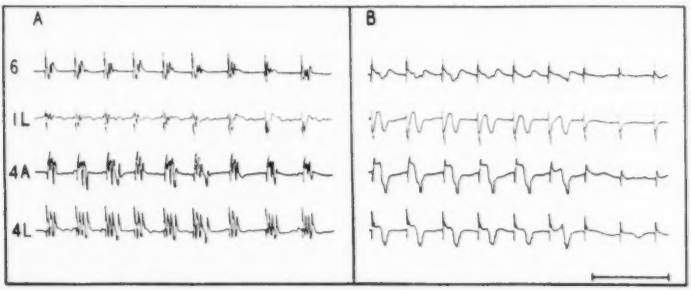

Fig. 2. One mode of ending of driven clonic responses. Records: *6*, *1*L, *4*A, and *4*L. Rapid stimulation of *4*A initiated tonic-clonic activity which spread to all of the recording regions. Single shocks were applied to *8* at the frequency shown by the stimulus artifacts (see end of B). These shocks controlled the clonic discharges. A illustrates typical responses early in the series. In B (40 sec. later) the responses have lost the spike components.

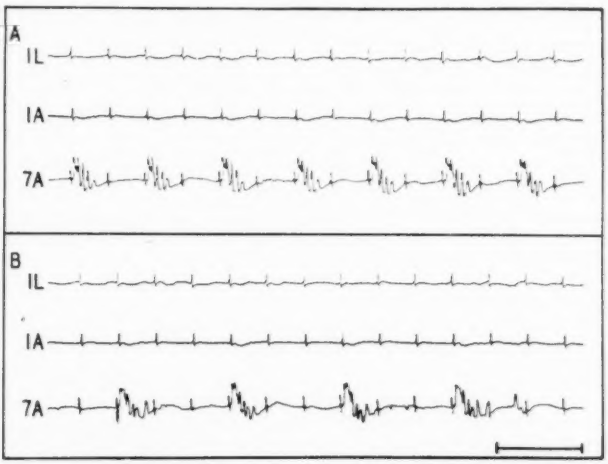

Fig. 3. Another mode of ending of driven clonic responses. Records: *1*L, *1*A and *7*A. Localized tonic-clonic activity was initiated in *7*. The stimulus artifacts denote single-shock stimulation of *6*. In A the clonic bursts bear a 1:2 ratio with the stimuli. In B, 10 sec. later, the ratio is 1:3. The last burst in the record was the last clonic response evoked.

quency of the prolonging stimuli was more effective if strong and long initial rapid stimulation had been applied, thus causing a widespread tonic-clonic response, than it was if weak and brief initial stimulation caused only localized selfsustained activity.

The end of clonic activity in response to single shocks of an appropriate fre-

quency could take place in one of several manners. First, with regard to rate, the clonic bursts could follow each single shock to a sudden abrupt end of the response (fig. 2), or else, after having followed a 1:1 relation with the stimuli for some time, they could begin to alternate so that they bore a 1:2, or, later, a 1:3 relation with the shocks, until they finally stopped (fig. 3).

Second, the latency of the clonic bursts elicited by the single shocks was sometimes constant until the responses disappeared (fig. 3). Sometimes, however, toward the end of a series a gradual increase of latency was seen (fig. 2).

Third, with regard to phase or pattern, the clonic responses sometimes remained quite constant until an abrupt end (fig. 3). Not uncommonly, however, there was a progressive simplification of pattern—the number of spike compo-

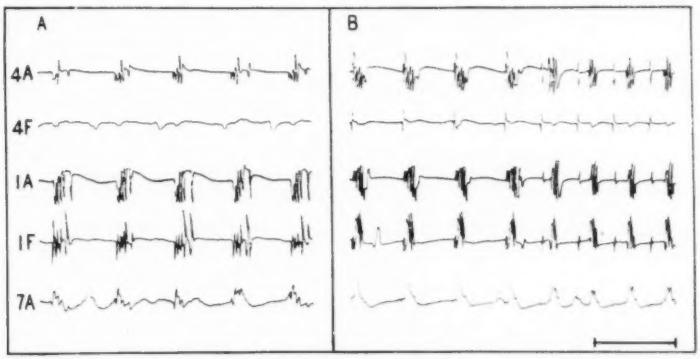

Fig. 4. Influence of rate of controlling single shocks on the clonic responses. Records: 4A, 4F, 1A, 1F, and 7A.

A. Illustrates clonic bursts in a selfsustained (undriven) response to rapid stimulation of 1A.

B. A rapid stimulus similar to that in A was applied. In addition single shocks were delivered to 6A, as shown by the stimulus artifacts (see tracing of 4F). The record illustrates the result of a sudden increase of the frequency of the single shocks: the latency of the clonic responses increases and the number of spikes in each burst decreases.

nents of the clonic complex decreased, while the slower smooth-wave component could remain unaffected or even increase in amplitude (fig. 2).

The pattern of the clonic bursts could also be affected by the rate of the controlling shocks. A characteristic example is illustrated in figure 4B. An acceleration of the driving stimuli resulted in a sudden decrease of the number of spikes in the bursts, and in addition caused an increase of the latency of the responses.

B. *Specific connections between different cortical areas.* In the previous section were considered the general characteristics of the clonic bursts evoked by single-shock stimulation of appropriate cortical areas. In this section the emphasis is on what is meant by appropriate in that context.

When a localized (non-spreading) tonic-clonic response was initiated in a given cortical region it was found that stimulation of certain other areas could control

and prolong the clonic bursts, whereas single-shock stimulation of still other areas would fail to influence the clonus.

The complete knowledge of controlling relationships in the cortex would require a study of all the regions controlled by any given area and of all the regions which can control that area. Such study was not attempted. Enough observations were made, however, to warrant the statement of the following principles.

a. There are pairs of areas in which mutual, two-way control is found. Thus, stimulation of area *4* (Brodmann, 1905) can drive clonus at area *7*, and *vice versa*.

b. There are pairs of areas which exhibit only one-way control. Thus, stimulation of area *17* can drive clonus at area *8* (fig. 5A), but stimulation of *8* does not drive the clonic responses at *17*.

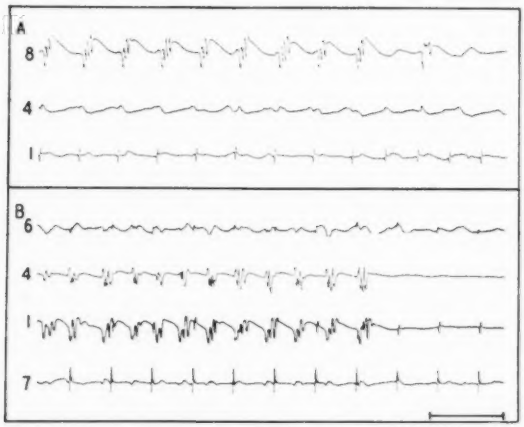

Fig. 5. Specificity of the controlling pathways.

A. Records: *8*, *4* and *1*. Single shocks to *17* (see artifacts in *1*) control a clonus localized in the distant area *8*.

B. Records: *6*, *4*, *1* and *7*. Single shocks to *19* (see artifacts in *7*) fail to control clonus at *4* and *1*, though *19*, like *17*, can control *8*.

c. There are pairs of areas with no mutual controlling relationships. Thus, stimulation of either *4* or *7* does not control a clonus limited to either *19* or *17*, and *vice versa* (see fig. 5B).

d. It is possible to set up 2nd or higher order control, thereby linking areas which are not directly coupled. Thus, as stated in *c*, *17* does not control *4*. Area *8*, on the other hand, controls *4*, and, as stated in *b*, *17* controls *8*. If tonic-clonic responses are set up both in *8* and *4*, stimulation of *17* may control clonus at *4* via *8*.

e. If single-shock stimulation of a given region controls the clonic activity of another, then the selfsustained clonic discharges in the first will also control the clonic activity of the second, provided that the rate of discharge of the driving area is faster than that of the driven. Figure 6 illustrates the control of clonic activity at *8* by the clonic bursts at *17* and *19*. In figure 7C, on the other hand,

19 fails to drive *8*, because the rate is slower at *19* than at *8*. Second order control is also possible in these conditions (see fig. 7D).

In addition to the controlling relationships already mentioned as examples, the following linkages were also observed. Areas *4*, *1*, *2* and *7* (cf. fig. 7B) all showed mutual control. This coupling was not limited to the "face", "arm" and "leg" bands which Dusser de Barenne and McCulloch (1938) described on the basis of the effects of local applications of strychnine. The face region of areas *4* or *1* could be readily driven by single shocks applied to the leg region (fig. 8), and *vice versa*, even if the arm region was not active during the tonic-clonic response.

Crossed couplings—i.e., from one hemisphere to areas on the opposite side—were also readily demonstrable. Thus, stimulation of *4* on one side controlled the clonic responses of *4*, *1*, *2* and *7* on the contralateral hemisphere.

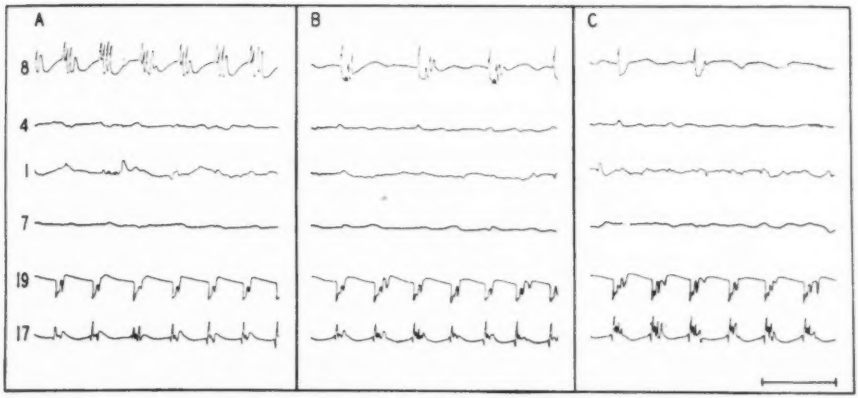

Fig. 6. Coupling of localized selfsustained clonic responses in distant areas. Records: *8*, *4*, *1*, *7*, *19* and *17*. Successive rapid stimuli were applied to areas *8* and *17*. A was taken 40 sec. after the application of the stimuli; for each clonic burst at *17* and *19* there is, with a brief delay, a corresponding clonic discharge at *8*. B, 10 sec. later; the discharges at *17* and *19* bear a 2:1 relationship with those at *8*—i.e., *8* is alternating with respect to the other areas. C, 10 sec. later; the response ends at *8* while it proceeds at *17* and *19*.

C. *The possibility of setting up two independent tonic-clonic responses in the cortex.* Since there are areas between which there is no mutual control, it was expected that separate discrete stimulation of such areas would result in responses independent of each other. This expectation was confirmed. For example, by successive stimulation of *7* and either *17* or *19*, it was possible to initiate responses with different clonic rates and durations (fig. 7A).

Even in areas with one-way coupling, independent responses could be obtained if the clonic rate of the driven area happened to be higher and the duration of the response longer than that in the driving area (cf. *19* and *8* in fig. 7C). The driving region could then not impose its rate on the driven one, much as single shocks fail to drive clonus when slower than the prevailing clonic rate (p. 682).

Occasionally, for reasons still obscure, independent responses were seen in areas usually coupled (cf. *8* with *4*, *1* and *7*, in fig. 7B). A linking of independent

areas could be easily obtained by simultaneous activity in a region coupled to the others (fig. 7D). The situation was then similar to that described above when second order controls were defined.

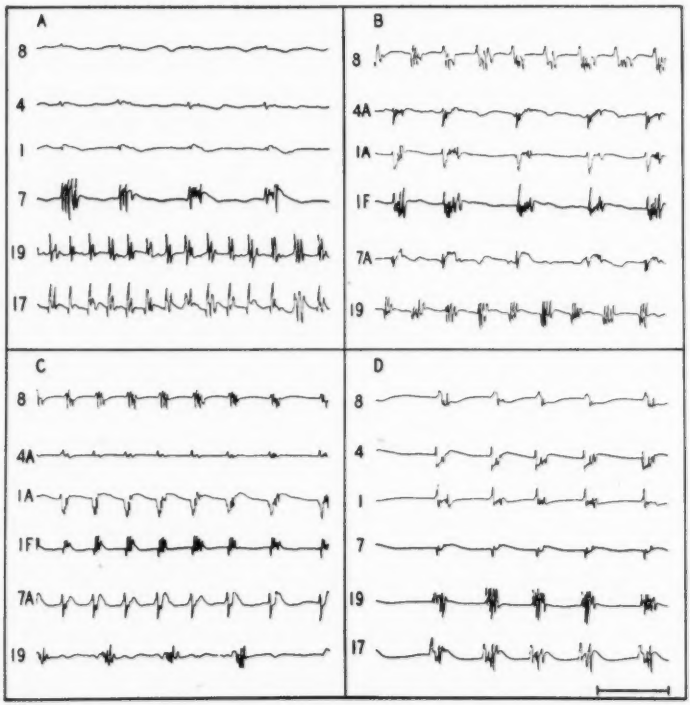

Fig. 7. Independent tonic-clonic responses in the cortex.

A. Records: *8*, *4*, *1*, *7*, *19* and *17*. Successive rapid stimulation at *17* and *7* led to two independent tonic-clonic responses, one at *7*, the other at *17* and *19*. The record shows the end of activity at *7*.

B. Records: *8*, *4*A, *1*A, *1*F, *7*A, and *19*. Rapid stimuli were applied first to *4*A and 15 sec. later to *8* and *19* in quick succession. The record begins about 20 sec. after the last stimulation. The clonus at *4*, *1* and *7* is coördinated and is independent of that at *8* and *19*.

C. Records as in B. Rapid stimulation was first applied to *19*. About 30 sec. later *4* and *9* were also stimulated. The record shows the end of the response at *19*. This response was independent of the coördinated discharges in the other areas.

D. Records as in A. Successive rapid stimulation of appropriate duration at *4*, *8* and *17* led to a clonic response which was coördinated throughout the cortex. The discharges at *17* lead; their control over the activity at *4*, *1* and *7* is made via *8* (second order control).

D. *The effects of sections of the gray matter.* The purpose of these observations was to see whether the controlling pathways are intracortical or subcortical.

In some instances a cut was made in areas *4* or *1*, about 5 mm. deep, from the lower margin of the face region up to the midline. Single shocks were then ap-

plied to *6* arm and a clonic response was stimulated in *7* arm. The single shocks invariably controlled the clonus at *7*, much as they had before the cut.

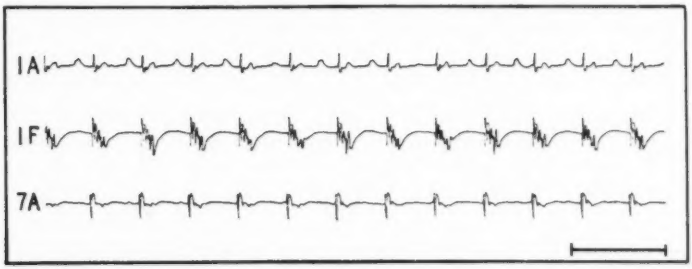

Fig. 8. Coupling of different divisions of a single area. Records: *1*A, *1*F and 7A. Rapid stimulation was applied to *1*F; it resulted in a tonic-clonic response which did not spread to the neighboring division *1*A or to other areas. The record shows the control of the clonic bursts at *1*F by single shocks applied to *1*L. Stimulus artifacts in *1*A and 7A.

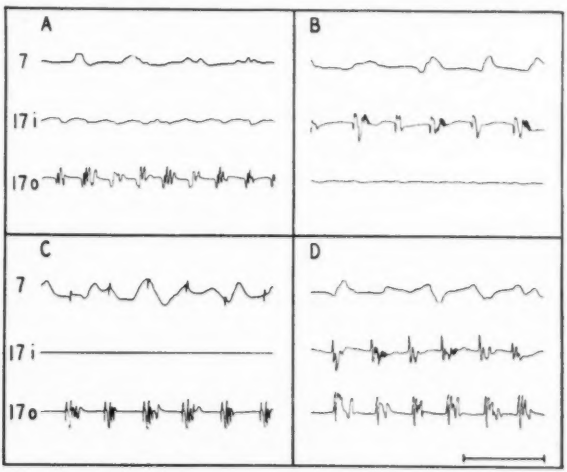

Fig. 9. Responses of an isolated cortical region in area *17*. As explained in the text, the gray matter was cut throughout the perimeter of a square with sides of about 2 cm. Records: *7*, *17* inside, and *17* outside the isolated region.

A. Rapid stimulation outside caused a tonic-clonic response which did not spread inside. The clonus is illustrated.

B. As in A, but stimuli applied inside.

C. Rapid stimuli as in A. Single shocks were then applied inside and, as shown by the record, succeeded in controlling the clonic response. Each clonic burst is preceded by a diphasic stimulus artifact.

D. Two successive rapid stimulations were applied, one inside, one outside. The record illustrates the synchronism of the selfsustained clonic bursts.

In other more conclusive cases a square of gray matter with sides of about 2 cm. was entirely isolated in area *17* by 3 sections about 5 mm. deep, that included

the pia, and by an additional subpial cut, where the vessels entered the area. Records were then taken from 2 pairs of electrodes, one outside, the other inside the isolated region. As shown in figure 9A and B, the response to rapid stimulation outside the cuts did not spread to the isolated portion, and similarly, stimulation inside did not spread beyond the sections. Single-shocks applied inside, however, readily controlled the clonic responses of the region outside (fig. 9C), and *vice versa*. Furthermore, if successive rapid stimulation was applied inside and outside, the clonic bursts were correlated at the two pairs of electrodes (fig. 9D).

E. *The control of clonic responses by afferent nerve impulses.* The fact that clonic responses may be driven by electrical stimulation of the cortex naturally

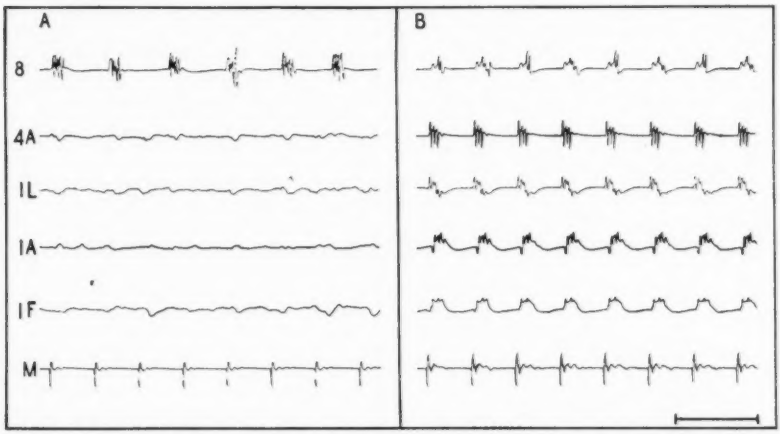

Fig. 10. Second order control in driving by afferent nerve impulses. Records: *8*, *4A*, *1L*, *1A* and *1F*. The lower tracing (M) records the action potentials of a muscle activated reflexly; it indicates the time of single-shock afferent stimulation of the right sciatic.

In A sciatic stimulation fails to drive a localized clonic response at *8*.

In B sciatic stimulation drives clonus at *8* because this area is now coupled to other active regions over which the afferent impulses have a control.

suggested the possibility that centripetal impulses, set up by stimulation of afferent nerves, might also control clonic activity.

Accordingly, in some animals, in addition to the usual cortical leads, stimulating electrodes were applied to the central end of the cut right sciatic nerve. It was possible for the afferent nerve impulses to gain control of the clonus at several areas, both in the contralateral (fig. 10B) and in the ipsilateral hemispheres. Second order control is illustrated in figure 10. Sciatic stimulation did not influence the clonic activity at the contralateral area *8* when this activity was localized (A). Adequate control ensued, however, when *8* was coupled to other controlled areas (B).

Discussion. I. *The similarity of the clonic responses to single shocks with the spontaneous clonic bursts.* Throughout the description of the experimental results the assumption was made that the responses obtained by appropriate single-

shock stimulation are entirely similar to the clonic discharges of a tonic-clonic response. This assumption is based on the following considerations.

a. The responses to the single shocks, like the clonic bursts, include several spikes and a more prolonged wave, organized in a characteristic pattern (cf. fig. 4A and B).

b. The single shocks, when applied before or some time (several seconds) after a tonic-clonic response, elicit either no recordable activity or only unsustained simple responses of the type previously studied by Adrian (1936) and by Rosenblueth and Cannon (1942). Such simple responses, when present, in no aspect resemble a clonic burst. Indeed, the single shocks elicit clonic-like effects only when delivered during the clonus of the tonic-clonic sequence, not before.

c. Single shocks delivered shortly after a spontaneous clonic burst fail to elicit any typical response (p. 682). This observation leads to the inference that the same cortical elements are involved in selfsustained clonus and in the responses to single stimuli.

d. Areas which couple upon single-shock stimulation also exhibit linked undriven clonic bursts when stimulated separately (fig. 6). Conversely, uncoupled areas show independent selfsustained clonic activity (fig. 7A).

e. The rates at which electric stimulation of one region may drive another coupled region are similar to those at which selfsustained clonus occurs (p. 683).

II. *The pathways for control.* The conduction of the nerve impulses from a driving stimulated area to a driven clonically active region could *a priori* follow two different pathways. The stimulated cells could activate neighboring elements, and these in turn more outlying neurons, so that a spreading wave of activity would ensue, largely confined to the gray matter. Such waves would follow—to use a word suggested by W. S. McCulloch (personal communication)—the "feltwork" of the cortex. This mode of propagation is exhibited by some of the cortical responses to single-shock stimulation studied by Adrian (1936) and by Rosenblueth and Cannon (1942).

An alternative pathway could be long fiber tracts, with few or no relays, traveling subcortically from the driving to the driven areas. This means of conduction would ensure specificity of connections, as opposed to the indiscriminate spread which would tend to result from feltwork propagation.

That the coupling of areas is probably through long, subcortical connections is indicated by the following observations. *a.* The control is specific (fig. 5). *b.* The coupling of areas in the two hemispheres (p. 687) is undoubtedly through long pathways. *c.* Quite distant areas in one hemisphere (e.g., *17* and *8*) are coupled and one may drive the other, although it may not drive some of the intermediate regions of the cortex (fig. 5). *d.* Propagation velocity in the feltwork is relatively slow (Adrian, 1936; Rosenblueth and Cannon, 1942); yet the latency of the clonic bursts elicited in *8* by stimulation of *17* was often as brief as 25 msec. *e.* Section of the gray matter between the driving and the driven regions did not abolish the linkages between different cortical regions (fig. 9; p. 688).

In a recent study, Le Gros Clark (1941) failed to obtain any anatomical evidence of long unilateral cortico-cortical connections of area *17*. The present data give no clue as to the anatomical structure of the coupling tracts. They

indicate, however, that there are specific, long, functional pathways linking certain areas with several others.

In this and a previous study (Rosenblueth and Cannon, 1942) the spread of a tonic-clonic response in one hemisphere has always been to contiguous areas, never discontinuous—i.e., a spread to a distant area has never been observed unless the intervening regions, between the stimulated and the distant areas, first became involved in the selfsustained activity. Thus, in the present observations, although areas *17* or *19* readily controlled the clonic activity of area *8*, stimulation of *17* or *19*, no matter how strong, frequent and prolonged, never caused the appearance of a tonic-clonic response in *8*.

Tonic-clonic responses of certain cortical areas in one hemisphere can be evoked by the stimulation of appropriate regions in the opposite side (Rosenblueth and Cannon, 1942). It may be inferred, therefore, that the activation of tonic-clonic responses may be brought about via long fiber connections. To reconcile this positive datum with the negative facts in the previous paragraph it is suggested that the long nerve paths involved in the spread of a tonic-clonic response from one to the other hemisphere are different from those through which control of clonus can be obtained. Within one hemisphere predominantly or exclusively controlling pathways are present, whereas the connections between the two hemispheres involve these controlling pathways and in addition others which lead to the spread of selfsustained activity. The spread of tonic-clonic activity within one hemisphere would then be predominantly or exclusively via the feltwork. This last suggestion is supported by the failure of spread across a cut of the gray in *17* (fig. 9).

III. *Some features of cortical clonic activity.* The data reveal some interesting properties of the cortical elements involved in clonic discharges. The latency of the clonic bursts of one area in response to stimulation of another one could vary within wide ranges. For example, in a single animal the latency of the responses at *8* to stimuli applied at *17* varied from 25 to 100 msec. The changes of latency could depend on the rate of the driving stimuli (fig. 4). They could occur progressively in the course of a series of responses (fig. 2B). Finally, the latency could vary with the intensity of the driving shocks—e.g., in one observation the latency at the left area *4* of the responses to single shocks at the right symmetrical region changed abruptly from 40 to 75 msec. when the intensity of the stimuli was reduced from 27 to 17 v.

Since the conduction velocity of axons is quite constant, it is not likely that the changes in latency are due to changes of conduction velocity in the pathway which connects the stimulated with the responding regions. It appears more probable that a lengthening of latency is due to a lengthening of the synaptic delays interposed in that pathway, particularly the synaptic delay at the driven area. This interpretation covers all the changes of latency observed if the further assumption is made that the synaptic delay in question varies inversely as the excitability of the responsive cells. The progressive lengthening of latency seen toward the end of a series of clonic responses (fig. 2) would then be due to a progressive decrease of the excitability of the activated elements. The longer latency corresponding to briefer intervals between the stimuli (fig. 4) would be

due to the relative refractoriness (p. 682) of the cells (i.e., lessened excitability), when the stimuli are delivered at short intervals. The increase of latency which results from a decrease of the intensity of the driving stimuli would not be due to a change of excitability of the responding cells but to a decrease of the "density of excitation." Weak shocks stimulate fewer controlling elements than do strong shocks. The total number of nerve impulses impinging at the active area is therefore small with a weak shock—i.e., the density of excitation is low.

Rosenblueth and Cannon (1942) described two components in the electrical records of cortical clonic bursts. One of them (component III) consists of sharp, spike-like excursions, commonly multiple in the bursts. The other one (component IV) is a large round wave, usually obscured by the superimposed spikes. The prolongation of a clonic response by a series of stimuli reveals that these two components correspond to activity of different and independent elements, since often the spikes disappeared in the course of the series while component IV was still present for some time, until a sudden or gradual disappearance (fig. 2).

Changes of amplitude and duration in the clonic bursts, correlated with the frequency of clonus, occur not only when this frequency is controlled (fig. 4) but are also seen in selfsustained (undriven) clonic responses. In the course of the selfsustained discharges the frequency slows progressively and the complexity of the bursts increases correspondingly (Rosenblueth and Cannon, 1942). In nerve and cardiac or striated muscle, impulses elicited in rapid succession are subnormal in amplitude, for recovery from previous activity is not complete. There is not a strict parallelism between these simple responses and the clonic bursts. A clonic discharge involves probably single activity of some elements (component IV), but it also includes repetitive trains in others (component III). Even for this complicated pattern, however, the responses appear simplified, if sufficient time for total recovery is not allowed.

IV. *The concept of a background excitation.* To clarify this concept we shall consider specific experimental instances. Single shocks applied to area *17*, without any other stimulation of the cortex, do not give rise to clonic bursts in the ipsilateral area *8*. The same shocks, however, when applied immediately after the end of a tonic-clonic response stimulated at *8*, regularly elicit further typical clonic bursts in this region. These clonic responses may then be evoked for a long time, if the stimuli to *17* are applied above a frequency of about 1 per sec. They then will stop suddenly even though the stimuli be continued. There is no evidence of any cortical activity at *8* between the bursts produced by stimulation of *17*; indeed, even the spontaneous activity, otherwise ever present in the cortex, may be absent at the time (see Rosenblueth and Cannon, 1942). It is suggested; *a*, that the stimulation at *8*, which initiates the tonic-clonic response, builds up a prolonged state of enduring excitation in some cells of that area; *b*, that this excitation is responsible for the clonic bursts during the selfsustained response; *c*, that the excitation gradually subsides; *d*, that the progressively decreasing frequency of the spontaneous clonic bursts indicates the gradual wane of excitation; *e*, that the selfsustained discharges cease when the excitation drops below a critical level; *f*, that there still remains, however, enough background excitation so that the impulses arising at *17* succeed in tripping the clonic elements at *8*; and

g, that the end of the driven clonic bursts again indicates further wane of excitation below a second critical level.

Since a faster frequency of stimulation at *17* prolongs clonic discharges at *8* more than does a slower frequency (p. 683), it is further suggested, *h*, that each clonic burst in turn increases the background excitation in the discharging and in other similar elements. In support of this suggestion is the fact that a long tonic-clonic selfsustained response at *17* prolongs a brief tonic-clonic response at *8*—i.e., that clonic discharges at *17* may drive clonic discharges at *8* (fig. 6).

V. *The end of selfsustained tonic-clonic responses.* Two questions arise with regard to the end of a tonic-clonic response that is not prolonged by single-shock stimulation: why does the response cease?; and why does it usually cease simultaneously (Rosenblueth and Cannon, 1942) at all the active areas?

The present observations, interpreted on the basis of the suggestions outlined in the previous section, furnish an answer to these questions. When a response does not spread beyond the stimulated region the discharges cease as soon as the background excitation reaches a sufficiently low level. When a response has spread, and several areas are involved, then although the excitation may have waned at some of the regions below the level necessary for automatic discharge, such regions will remain active because they are controlled by those where excitation is still high. As long as one of the areas, which controls directly or indirectly the others, has enough background excitation for clonic activity, the response will proceed throughout, but it will suddenly stop everywhere when excitation drops below the critical level in the last controlling area.

SUMMARY

In Rhesus monkeys, under chloralose anesthesia, tonic-clonic cortical responses were elicited by rapid electric stimulation. Single shocks applied to appropriate cortical areas can control the rate of the clonic discharges and can prolong the responses beyond their intrinsic duration.

The following features of the driven clonic responses are described: alternation (fig. 1); termination of a series (figs. 2 and 3); influence of frequency (fig. 4); pattern (figs. 2 and 4); specificity of the controlling connections (figs. 5 and 8). The controlling pathways are subcortical (fig. 9). Localized tonic-clonic responses may be coupled in distant areas (fig. 6); or else they may be independent (fig. 7).

Afferent nerve impulses can control clonic discharges (fig. 10).

The discussion deals with the similarity of the driven to the undriven clonic bursts (p. 690), the controlling pathways (p. 691), some properties of clonically active elements (p. 692), the background cortical excitation (p. 693), and the factors which determine the rate and end of a clonic selfsustained response (p. 694).

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THE EFFECT OF PERIPHERAL VASODILATATION ON VASOCONSTRICTION: DETERMINATIONS MADE ON THE BASIS OF BLOOD PRESSURE OF NORMAL SUBJECTS[1]

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Received for publication July 7, 1942

In normal subjects elevation of the blood pressure or a vasoconstrictor response to painful stimuli has been observed by many investigators. Hines and Brown (1) have demonstrated that after the basal blood pressure has been obtained, immersion of one hand to a point just above the wrist in water at 4°C. for one minute will produce a rise of 10 to 20 mm. of mercury in the systolic blood pressure and 8 to 15 mm. in the diastolic. General anesthesia inhibited this response, derivatives of barbituric acid markedly decreased the reaction, while bromides caused only a slight decrease in it. Except for the effect of these drugs, this vasoconstrictor response over various periods was remarkably constant.

Since, for clinical purposes, 95 per cent ethyl alcohol administered orally has been used to produce peripheral vasodilatation, the question arose as to whether such peripheral vasodilatation could inhibit or alter this vasoconstrictor response of the blood pressure in normal subjects. Cook and Brown (2) have demonstrated that following the oral administration of 0.5 cc. of 95 per cent ethyl alcohol for each kilogram of body weight, the degree of vasodilatation of the vessels of the skin of the extremities of normal subjects approached that obtained with anesthesia or fever. The average maximal level reached by the surface temperature of the skin of the toes was 33.1°C. It has also been shown that when a normal subject remained for an hour or more under a hot environmental temperature of 32°C. (89.6°F.) the temperatures of the fingers and toes closely approximated the temperatures of the forehead, thorax, legs and arms, thereby indicating more or less generalized vasodilatation of the peripheral blood vessels. In order to investigate whether 95 per cent ethyl alcohol, administered orally, produced adequate peripheral vasodilatation, exposure of the normal subject to an environmental temperature of 32°C. (89.6°F.) for approximately an hour with the accompanying rise of the skin temperature of the toes could be used as comparative evidence.

The present study was made to determine whether marked peripheral vasodilatation, produced either by the oral administration of 95 per cent ethyl alcohol or by exposure of the subject to an atmospheric temperature of 32°C. (89.6°F.) for an adequate period, could alter or inhibit the vasoconstrictor response of the blood pressure of normal subjects to a painful stimulus. Since more or less generalized vasodilatation of the peripheral circulation is considered to be present

[1] Read before the meeting of the American Physiological Society, Boston, Mass., March 31 to April 4, 1942.

when the temperature of the skin of the toes approximates that of the skin of the fingers, and since the skin temperature of the toes is the most sensitive or delicate indicator of the vasomotor regulation of the dissipation of heat, various criteria must be maintained regarding posture, constancy of the environmental temperature and the basal state of the subject. Furthermore, higher skin temperatures of the toes are found in persons who have higher basal metabolic rates.

PROCEDURE. Data were obtained in psychrometric rooms on twelve normal subjects whose ages ranged from eighteen to forty years. The studies with alcohol took place in a room where the temperature was maintained at 25.5°C. with a relative humidity of 40 per cent. The subjects were fasted for fifteen hours previous to the tests and during the tests they wore lightweight short pajamas and were in the supine position on comfortable beds. The basal metabolic rates were first determined. Basal blood pressures were then observed and one hand was immersed in water at 4°C. for one minute and the rise of blood pressure was noted during the immersion. After the skin of the hand had returned to normal temperature, the temperatures of the plantar surfaces of the first and third toes of both feet and of the volar side of the distal phalanges of the first and third fingers of the two hands were measured by means of copper-constantan thermocouples. When fairly constant readings of the skin temperatures of the extremities were obtained, 30 cc. of 95 per cent ethyl alcohol diluted in 150 cc. of fruit juice, which was equivalent to 0.5 cc. per kilogram of body weight, was then administered orally and the blood pressure and skin temperatures were observed at ten minute intervals for one to two hours. When the skin temperature of the toes approximated that of the fingers, the same hand previously used was again immersed in water at 4°C. for one minute to determine the vasoconstrictor response of the blood pressure in the presence of dilatation of the peripheral blood vessels.

At some later time in eight of the subjects, the vasoconstrictor response of the blood pressure was again determined and the subject was placed for one to two hours in a hot room where the environmental temperature was maintained at 32°C. (89.6°F.) with a relative humidity of 40 per cent. The skin temperature of the fingers and toes was again determined and observations of blood pressure were made at intervals of ten minutes. Again, when the skin temperature of the toes approximated that of the fingers, the vasoconstrictor response of the blood pressure was repeated.

RESULTS. The basal metabolic rates of these twelve normal subjects ranged from +13 to −19 per cent. Under an environmental temperature of 25.5°C. with a relative humidity of 40 per cent, as previously mentioned, the highest skin temperature of the toes was observed among subjects who had the highest basal metabolic rates.

The average skin temperature of the toes for the group during the control period was 26.9°C. with a range from 22.5 to 31.3°C. while the average skin temperature for the fingers during the same period was 31.2°C. with a range from 27.1 to 34.5°C. The average difference between the fingers and toes was 4.3°C.

Approximately one hour after the oral administration of 30 cc. of 95 per cent

ethyl alcohol, the average maximal skin temperature of the toes was 31.8°C. with a range from 23.6 to 35.3°C. while the average maximal skin temperature of the fingers was 34.2°C. with a range from 30.3 to 36.0°C. In spite of the seeming disparity between skin temperatures of the fingers and toes, only in three instances was there a difference of 2°C. or more.

After the subjects had remained in the hot room for one hour generalized peripheral vasodilatation was evident when the skin temperatures of the toes reached the average maximal temperature of 34.2°C. with a range from 32.6 to 35.5°C. while the fingers reached an average maximum of 35.9° with a range from

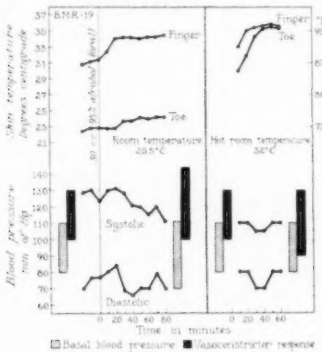

Fig. 1

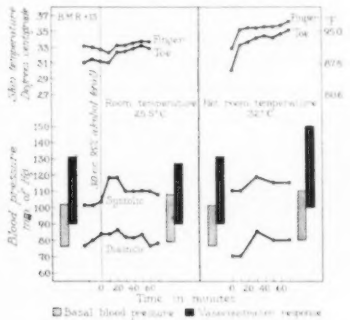

Fig. 2

Fig. 1. With a basal metabolic rate of −19 per cent, the skin temperature of the toes was about 3°C. below the room temperature and one and a half hours after the administration of ethyl alcohol it did not approximate that of the fingers. A difference of more than 10°C. existed between the skin temperatures of the fingers and toes. After the subject had been exposed to the high environmental temperature, the temperature of the skin of the toe approximated that of the finger. The vasoconstrictor response of the blood pressure was slightly higher after the administration of ethyl alcohol in this subject than with the high environmental temperature.

Fig. 2. With a basal metabolic rate of +13 per cent the skin temperature of the toes and the fingers was considerably higher than room temperature and the temperature of the toes readily approximated that of the fingers with both the administration of ethyl alcohol and subjection to a high environmental temperature. In this subject, the vasoconstrictor response of the blood pressure was slightly higher with the hot environmental temperature than after administration of ethyl alcohol.

35.4 to 36.1°C. The average maximal temperatures were slightly higher under the increased environmental temperatures than with 95 per cent ethyl alcohol.

During the control period, the average basal blood pressure in millimeters of mercury for the group was 104.3/73, with a range of 90/50 to 120/94. During the immersion of one hand up to the wrist in water at 4°C. for one minute the blood pressure rose to an average in millimeters of mercury of 124/88 with a range of 102/74 to 140/100. The average increase was 19 mm. of mercury in the systolic and 15.4 mm. in the diastolic.

Approximately one hour after the oral administration of 30 cc. of 95 per cent ethyl alcohol when the temperature of the skin of the toes demonstrated more or

less generalized peripheral vasodilatation, the average basal blood pressure in millimeters of mercury for the group was 101.6/71 with a range from 90/55 to 122/90. Immediately following this observation, the hand was immersed in water at 4°C. for one minute. During immersion the average blood pressure rose to 121/87 with a range from 94/70 to 144/100. The average increase was 20.7 mm. of mercury in the systolic and 16.5 mm. in the diastolic. This response of the blood pressure was similar to that produced during the control period. At another time the average basal blood pressure in millimeters of mercury of eight of the subjects, during a control period, was 102.2/69 with a range from 90/55 to 110/75. After exposure of the subjects to an environmental temperature of 32°C. (89.6°F.) for between one and two hours and when the temperature of the skin of the toes demonstrated generalized peripheral vasodilatation the average basal blood pressure for the group was 98.8/66.3 mm. of mercury, with a range of 90/55 to 110/80. The hand was immersed again in water at 4°C. for one minute, and during immersion the blood pressure in millimeters of mercury rose to an average of 123.9/87.0 with a range of 108/75 to 150/100. The slight changes which took place could not be considered significant.

A composite picture of some of the variations of the skin temperatures and blood pressures is given for two subjects (figs. 1 and 2), one with a basal metabolic rate of −19 per cent and the other with a basal metabolic rate of +13 per cent.

SUMMARY

In these twelve normal subjects, irrespective of the basal metabolic rate and irrespective of the existing generalized peripheral vasodilatation, the response to the vasoconstricting agent was not altered significantly.

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THE PRODUCTION OF EXPERIMENTAL POLYCYTHEMIA BY THE DAILY ADMINISTRATION OF EPINEPHRINE OR POSTERIOR PITUITARY SOLUTION[1]

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Received for publication July 8, 1942

We have previously reported the production of experimental polycythemia in dogs, rabbits and man by the daily administration of appropriate doses of ephedrine sulfate. (1) Amphetamine sulfate was also found to be effective in augmenting erythropoiesis in dogs (1) and in man (2). The results were attributed to local hypoxia of bone marrow due to the curtailment of its blood supply through the vasoconstrictor action of these drugs.

The work to be reported here was performed in an effort to determine whether other well known vasoconstrictor drugs could produce polycythemia, when given daily in appropriate doses. Epinephrine and solution of posterior pituitary were the drugs selected for this experiment. It is known that polycythemia often accompanies human cases of pituitary basophilic adenoma, and it is possible that this polycythemia might be due to an associated hyperactivity of the adrenal medulla. High doses of pituitrin have been shown to cause experimental "pituitrin anemia" in rabbits (Dodds et al., 3, and Gilman and Goodman, 4), which is due to hemolysis of red cells caused by dilution of the serum electrolyte as a result of water retention.

PROCEDURE. Dogs and rabbits were used in this investigation. They were maintained on constant adequate diets and were allowed water ad libitum. Control observations were made on the blood of each animal during a period of 3 weeks prior to drug administration. Red cell counts were made regularly together with estimations of hemoglobin percentage (Hellige). Total leukocyte counts were also made at intervals.

Solution of posterior pituitary was injected subcutaneously into one splenectomized and two normal dogs in daily doses of 5 and 10 units. The same preparation was given to 4 normal and 2 splenectomized rabbits in daily subcutaneous doses of 0.5, 1.0 and 2 units, for about 15 days. Epinephrine hydrochloride was injected subcutaneously into one splenectomized and two normal dogs in daily doses of 0.5, 1.5 and 2 mgm. Epinephrine was also injected daily into 3 normal and 2 splenectomized rabbits in doses ranging from 0.1 to 0.3 mgm.

During the experiment, blood samples were drawn only after an interval of at least 18 hours following the last drug injection. Precautions were taken to keep the animals as free from excitement as possible. Blood was drawn by syringe from the external saphenous veins of the dogs; in the rabbits, blood was drawn directly into diluting pipettes from the site of puncture of a marginal ear vein.

[1] Research paper no. 531, journal series, University of Arkansas.

After about 18 days the drug administrations were stopped, and in most instances observations on the blood were continued for about 2 weeks thereafter.

RESULTS. The daily subcutaneous injection of 0.5 to 2 units of solution of posterior pituitary into 4 normal and 2 splenectomized rabbits resulted in significant increases (11 to 20 per cent) in their basal erythrocyte numbers within 9 to 14 days (fig. 1). The animals receiving the lowest dose of posterior pituitary (0.5 unit) showed the least increase of red cells. The counts returned to normal in 10 to 15 days following the cessation of drug injections.

The red cell counts of one splenectomized and two normal dogs were increased by 15 to 21 per cent by the daily subcutaneous injection of 5 to 10 units of solution of posterior pituitary for 10 to 17 days (fig. 2). Hemoglobin percentages increased correspondingly, but the total leukocyte counts did not change significantly. The erythrocyte counts in two of the dogs returned to normal in about 10 days following cessation of drug administrations. Unfortunately, the

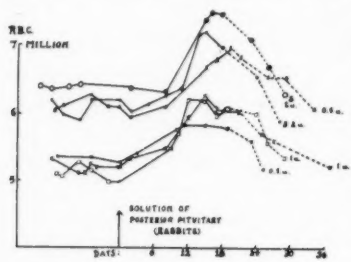

Fig. 1

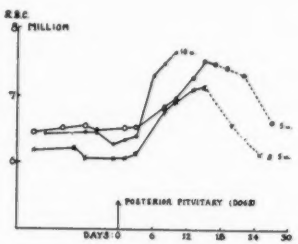

Fig. 2

Fig. 1. The effect of posterior pituitary solution on four normal and two splenectomized rabbits. *S* indicates splenectomized rabbit. Values at the end of each curve give the daily subcutaneous dose in units for each animal. Dash lines indicate cessation of drug administration.

Fig. 2. The development of polycythemia by dogs receiving posterior pituitary solution subcutaneously. Values at end of each line indicate the daily dose of drug for that particular animal (*u* = units). Dashes indicate discontinuation of drug injections. *S* indicates a splenectomized dog.

third dog (fig. 2), while very healthy, was accidentally allowed to escape from the animal house, and we were therefore unable to study the return of his erythrocyte count to normal.

Epinephrine hydrochloride was injected subcutaneously into 3 normal and 2 splenectomized rabbits in daily doses ranging from 0.1 to 0.3 mgm. As may be seen in figure 3, this procedure caused gradual increases in their erythrocyte numbers which became maximal after 12 to 18 days of drug injection. After cessation of epinephrine administration, the red cell counts returned to normal within about 15 days.

Figure 4 shows the development of polycythemia in one splenectomized and 2 normal dogs which received daily subcutaneous injections of epinephrine hydrochloride. The doses given were 0.5, 1.0 and 1.5 mgm., daily. After 15 days of drug injections, these animals showed increases in their red blood cell counts of 12 to 21 per cent. The dog which received the highest daily dose of

epinephrine showed the least increase of red cells. Hemoglobin percentages changed correspondingly with the erythrocyte count, but total leukocyte counts showed no uniform or constant change. Red cell counts in all 3 dogs returned to normal within 10 to 15 days after cessation of epinephrine administration.

Discussion. The results indicate that epinephrine and posterior pituitary probably caused an increased erythropoiesis in these experiments. Our strongest reason for this belief is to be found in the slow development of polycythemia (figs. 1–4) and the slow recovery from the same after discontinuation of the drugs. The time relationships correspond generally with those involved in the production of polycythemia by exposure to low atmospheric pressure (5).

The delay in production and recovery from polycythemia also argues against the possibility that our results might be due to blood concentration or to contraction of blood reservoirs. The relative constancy of the total leukocyte counts observed in our experiments on dogs constitutes additional evidence

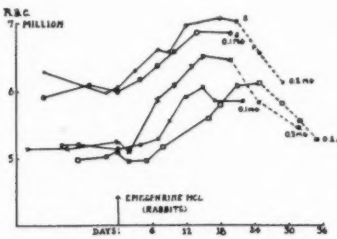

Fig. 3

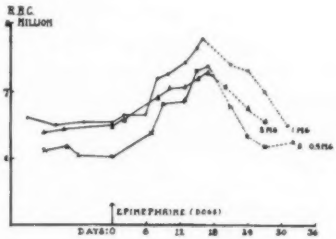

Fig. 4

Fig. 3. The production of polycythemia in rabbits by the daily subcutaneous injection of epinephrine hydrochloride. *S* indicates splenectomized animals, and values at the end of each line show the daily dose of drug for each rabbit. Dashes signify discontinuation of drug injections.

Fig. 4. The effect of daily subcutaneous injections of epinephrine on the erythrocyte numbers of dogs. *S* indicates a splenectomized dog. Values at end of each line indicate the daily drug dose. Dashes signify discontinuation of drug administration.

against the possibility of concentration of the blood. The fact that polycythemia is induced as readily in splenectomized as in normal animals (figs. 1–4) also suggests that blood reservoirs probably are not concerned in the development of polycythemia caused by epinephrine or posterior pituitary.

The most likely mechanism by which epinephrine and posterior pituitary increase hemopoiesis is probably through the creation of a local hypoxia of bone marrow. This would probably be brought about through diminution of the blood supply to the marrow by the vasoconstrictor action of the drugs.

It is perhaps permissible to speculate that the results of these experiments may explain the mechanism of the polycythemia which is sometimes observed in pituitary basophilism or Cushing's disease in humans.

CONCLUSIONS

The daily subcutaneous administration 0.5 to 2.0 units of posterior pituitary solution to four normal and two splenectomized rabbits caused significant

increases in their erythrocyte numbers within 9 to 14 days. One splenectomized and two normal dogs also showed significant polycythemias after the subcutaneous administration of 5 to 10 units of posterior pituitary solution, daily, for 10 to 17 days.

The daily subcutaneous injection of 0.1 to 0.3 mgm. of epinephrine hydrochloride into 3 normal and 2 splenectomized rabbits resulted in gradual increases in their erythrocyte counts which became maximal after 12 to 18 days. One splenectomized and two normal dogs also developed significant polycythemias within about 15 days following the onset of daily injections of 0.5 to 1.5 mgm. of epinephrine hydrochloride.

These results are explained by assuming that epinephrine and posterior pituitary cause increased erythropoiesis by inducing a local hypoxia of bone marrow. This is presumably accomplished through reduction of the blood supply to the marrow as a result of the vasoconstrictor action of these drugs.

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THE EFFECT OF ANOXIA ON BRAIN POTENTIALS OF HYPERTHYROID ANIMALS

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Received for publication July 9, 1942

The effect of thyroid administration on the oxygen uptake of excised brain is well established (Cohen and Gerard, 1937; Rossiter, 1940). Several authors (Streuli, 1918; Barach, Eckman and Molomut, 1941) have shown that thyroid administration increases and thyroidectomy decreases the sensitivity of various laboratory animals to anoxia. Therefore, it seemed worthy to investigate the effect of thyroid hormones on the electroencephalographic changes induced by anoxia.

METHODS. The experiments were performed on unanesthetized rats of approximately 250 grams weight. One group of the animals was injected with 0.1 to 0.2 mgm. thyroxine/100 gram weight for 9 days. Another group received 0.2 mgm. thyroxine/100 gram weight for 4 days. The third group was given thyroid powder U. S. P. (Armour and Co.) 100 mgm. per day for 12 days. Whereas the thyroxinized rats lost some weight it was not appreciably altered in the rats which were treated with thyroid powder. Anoxia was produced either by allowing the rats to inhale seven per cent oxygen from Douglas bags or by exposure to lowered barometric pressure of 280 and 255 mm. Hg. The E. E. G. was recorded using phonograph needle electrodes (Hoagland) through an Offner crystograph.

RESULTS. It has been pointed out in an earlier study (Gellhorn and Kessler, 1942) that no significant changes in E. E. G. are observed when normal rats are exposed to 7 per cent oxygen. However, thyroxinized rats (fig. 1) show profound changes in the E. E. G. under similar conditions. In the first experiment of figure 1 alpha potentials disappear more or less completely and slow delta waves appear. This effect is reversible on readmission of air. In the second experiment in which the control shows some delta waves the administration of 7 per cent O_2 greatly accentuates the delta potentials. Then the alpha potentials decrease in size and finally the brain waves disappear almost completely. In this case also the phenomenon is reversible.

The experiments involving the administration of thyroid powder were less effective than those performed with thyroxine. However, definite quantitative differences appeared between control and hyperthyroid animals. In 8 control experiments only two animals showed a definite transient increase in delta potentials at the lowered barometric pressure whereas the remaining 6 animals did not show any significant changes in the E. E. G. In the experimental group all animals showed on exposure to lowered pressure electroencephalographic changes which consisted either of a transient or a progressive increase in

[1] Aided by the John and Mary R. Markle Foundation.

delta potentials or of a complete disappearance of brain waves. This shows that electroencephalographic changes were more frequent and more severe in the experimental than in the control group.

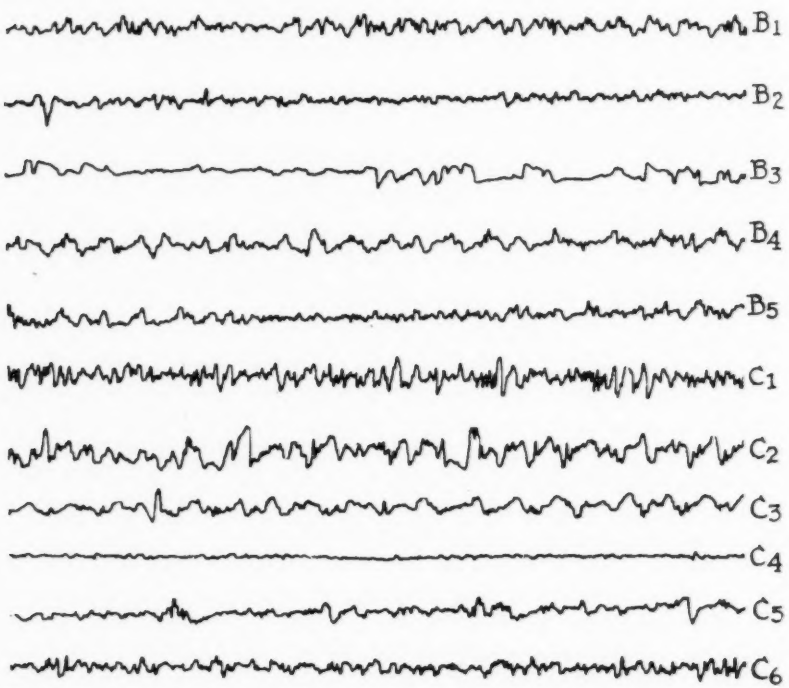

Fig. 1. The effect of 7 per cent O_2 on the E. E. G. of unanesthetized rats injected with thyroxine. B_1 control; B_2 and B_3 after one and three minutes of 7 per cent O_2 respectively. B_4 and B_5 1 and 4 minutes after readmission of air.

C_1 control in air; C_2 and C_3 after $2\frac{1}{2}$ and 3 minutes of 7 per cent O_2.

C_4, C_5 and C_6, 2, 3 and 9 minutes after readmission of air.

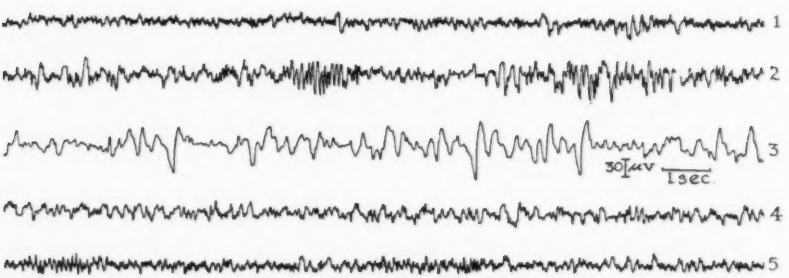

Fig. 2. The effect of lowered barometric pressure (280 mm. Hg) on the E. E. G. of rats which were given thyroid powder by stomach tube.

1, control at normal barometric pressure; *2*, *3*, *4*: 1, 7 and 12 minutes at 280 mm. Hg; 5, 10 minutes after readmission of air.

Figure 2 shows a marked increase in delta potentials accompanied by a decrease in alpha potentials at 280 mm. Hg. It is interesting to note that these effects were only transient (cf. records 3 and 4 of fig. 2) whereas the changes induced by anoxia (fig. 1) in thyroxinized rats were always progressive.

Apparently the sensitivity to anoxia as demonstrated by electroencephalographic changes is related to the degree of hyperthyroidism since anoxia produced the greatest changes in rats which had suffered loss of weight from the administration of thyroid hormones.

It should be added that the E. E. G. of the hyperthyroid animals at normal oxygen tension was unchanged.

SUMMARY

The administration of thyroid powder or of thyroxine increases the sensitivity of unanesthetized rats to 7 per cent O_2 or lowered barometric pressure as shown by the greatly accentuated effects of anoxia on the electroencephalogram.

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THE INFLUENCE OF ANTACIDS UPON IRON RETENTION BY THE ANEMIC RAT

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Received for publication July 10, 1942

An efficient utilization of dietary factors concerned in hemoglobin formation is of particular importance to the individual with a chronic ulcer of the stomach or duodenum. Such lesions may result in an excessive blood loss and some of the diets used are low in substances contributing to hemoglobin formation. Antacids are frequently administered in relatively large amounts to this group of patients, and it is possible that certain antacids may affect the absorption of iron from the intestine.

Kletzein (1) has shown that calcium carbonate as well as various other carbonates reduce iron retention in the anemic rat. He suggested (2) that the Ca-P ratio in the diet might be an important factor in influencing iron absorption. Anderson, McDonough and Elvehjem (3) studied the effect of various Ca-P ratios in the diet upon iron absorption by anemic rats and found that a low ratio favored iron absorption while a high Ca-P ratio resulted in a low assimilation of iron. A previous report (4) from this laboratory suggested the likelihood of aluminum hydroxide contributing to the anemia observed in dogs subjected to the Mann-Williamson operation. Data were presented at the same time which showed that aluminum hydroxide interferes with the absorption of phosphorus in dogs as well as in human subjects maintained on a "light ulcer diet."

The present study was undertaken to determine the effect of some "antacids" commonly employed in the treatment of chronic gastric and duodenal ulcer upon iron retention by the anemic rat. The relative dosage of the various anti-acids studied was chosen arbitrarily on the basis of what seems to be commonly employed comparable therapeutic doses.

EXPERIMENTAL PROCEDURE. *Animals.* Wistar strain rats were made anemic according to the method of Elvehjem and Kemmerer (5). When the animals were definitely anemic (hemoglobin 2–3 grams), each litter was divided into two groups with equal distribution of sexes and so that the average hemoglobin content of each group was similar. All animals were continued on a milk diet but the main daily feeding was withheld until all supplements were completely consumed. Both the control and the ant-acid-fed group received daily 0.25 mgm. iron as ferric chloride, 0.05 mgm. of copper and of manganese as their sulphates. These supplements were fed in a small quantity of milk. Aluminum hydroxide and aluminum phosphate were fed as a 5 per cent and 4 per cent suspension, respectively; the suspension was mixed with an amount of milk that the animal was certain to consume and the control group was likewise restricted. Calcium carbonate and magnesium trisilicate were fed as a dry powder mixed with finely

powdered cane sugar. The control group received an equal amount of cane sugar. All antacids used were shown to contain insignificant quantities of iron. The relative amounts of the various antacids fed were determined from the following considerations: a fairly liberal antacid regimen for an ulcer patient might require 200 cc. of 5 per cent aluminum hydroxide daily, 250 cc. of 4 per cent aluminum phosphate, or 6 grams of either calcium carbonate or magnesium trisilicate. The average iron requirement for man is considered to be in the vicinity of 10 mgm. daily. Then a comparable intake of iron and antacid would be in these proportions. Twenty-five hundredths milligram of iron daily has been shown (6) to cause marked increase in hemoglobin in the anemic rat. This amount does not represent an excess of iron and the organism is likely to utilize it as efficiently as possible. This amount of iron is approximately one-fortieth the daily dosage employed for man, e.g., 5 cc. of aluminum hydroxide, 6 cc. of aluminum phosphate, and 0.15 mgm. of calcium carbonate or magnesium trisilicate.

Each animal was caged separately. The cage consisted of an inverted bottomless 3-gallon bottle with a platform made of no. 2 mesh galvanized wire that was heavily tinned after construction of the platform. The platform had a diameter of 7.75 inches. Each cage had a wooden lid with numerous small perforations covered with gauze so that the cage is essentially fly-proof. This cage has the advantages ascribed to both glass and metal cages, namely, little chance for iron contamination or for refection to occur.

Methods. Hemoglobin was converted to alkaline hematin, according to the method of Wu (7) and read in a Klett-Summerson photoelectric colorimeter using filter no. 54. The white cell pipette used for measuring the blood for all determinations of hemoglobin was standardized on a sample of dog's blood whose hemoglobin content was calculated from its iron content (8). Inorganic phosphorus was estimated by Bodansky's modification (9) of the Kutner-Lichtenstein procedure (10).

The extent of depletion was indicated by the hemoglobin content of the blood. After depletion the animals were maintained on the supplements for 26 to 28 days and then killed by ether inhalation. The entire carcass was ashed in a muffle furnace, at 1000°F., dissolved in hydrochloric acid, and evaporated to dryness twice, redissolved in hydrochloric acid, and then made up to a definite volume with iron-free water and analyzed for iron (8). The color was estimated in a Klett-Summerson photoelectric colorimeter using filter no. 54 and a series of standard solutions. One group of animals was killed and analyzed at the end of the depletion period.

Experimental results. The results show (table 1) that the depleted rats contain approximately 1 mgm. of iron per rat. After 26 to 28 days on a daily supplement of 0.25 mgm. of iron, the entire carcass of the control group averaged from 4.26 to 4.56 mgm. per rat. There is close agreement between the various control groups. The antacids that most definitely reduced iron retention were aluminum hydroxide and calcium carbonate. The difference between the iron content of the control and experimental groups for either of these antacids is

quite consistent and striking. The critical ratio of the difference in iron content of the aluminum hydroxide-fed group and its control group is statistically significant. The same is true of the calcium carbonate-fed group. The hemoglobin content of the blood at the end of the experiment is in satisfactory agreement with the iron content of the carcass for the various groups and conveys the same impression as one derives from results on iron content. The iron content of the magnesium trisilicate-fed group is somewhat lower than that of the control group and the critical ratio indicates that the difference may be significant. The hemoglobin increase in this group was also less than for the control group. Aluminum phosphate causes no decrease in iron retention or in hemoglobin formation.

TABLE 1

SUBSTANCE	NUMBER OF ANIMALS		WEIGHT OF ANIMALS		WEIGHT INCREASE			TOTAL MGM. OF IRON PER RAT			AVE. INIT. Hb IN GMS./100 CC.	Hb INCREASE IN GMS./100 CC.			IRON RETN. PER CENT OF IRON FED
	Male	Female	Ave. init. wt.	Ave. final wt.	Mean in gms.	Stand. dev.	Crit. ratio	Mean in mgms.	Stand. dev.	Crit. ratio		Mean in gms.	Stand. dev.	Crit. ratio	
Aluminum hydroxide	7	3	86.5	131	44.5	4.44		2.97	0.156		2.60	2.92	0.323		30.6
							0.014			7.678*				6.755*	
Control	6	4	83	128	44.6	4.51		4.26	0.187		2.72	5.71	0.260		49.0
Aluminum phosphate	4	6	84	146	61.2	4.41		4.52	0.190		2.69	5.18	0.421		52.3
							2.337*			0.154				0.502	
Control	3	7	75	124	48.6	3.10		4.56	0.176		2.93	4.76	0.723		53.6
Calcium carbonate	3	8	63	133	69.5	5.13		3.43	0.090		2.49	4.79	0.293		37.0
							0.780			5.398*				4.195*	
Control	3	9	62	126	64	4.83		4.38	0.151		2.54	6.46	0.272		51.0
Magnesium trisilicate	4	7	61	117	56	4.99		3.90	0.231		2.84	5.61	0.292		43.8
							1.687			2.172*				1.519	
Control	4	7	62	130	67	4.195		4.50	0.152		2.86	6.16	0.216		52.8
Depleted group	6	4	75					0.94			2.60				

* Indicates significance as determined from J. P. Guilford's *Psychometric methods* (1st ed., McGraw-Hill, 1936). Significant critical ratios are indicated in table K, p. 548. Statistical method is described on pp. 44–51 inclusive.

Discussion. The results obtained in the present experiments are in agreement with the observations mentioned in the introduction. Fifteen-hundreths of a gram of calcium carbonate daily is enough calcium to alter the ratio of calcium to phosphorus in the diet of rats consuming from 30 to 60 cc. of milk daily from 1.2 to approximately 2 or 3 depending upon the amount of milk consumed. Aluminum hydroxide ingestion will increase the calcium-phosphorus ratio by reducing the available phosphorus. The extent to which this reaction will occur depends upon a number of variable factors (4). That the effect upon phosphorus absorption was not sufficient to deplete definitely the organism is indicated by the fact that the phosphorus content of four aluminum hydroxide-fed rats varied from 0.650 to 0.680 gram per 100 grams of carcass, averaging 0.663 gram, while four litter mate controls ranged from 0.670 to 0.727 gram per

100 grams of carcass with an average value of 0.694. However, it is the ratio of calcium-to-phosphorus in the intestine which seems to be of importance rather than the absolute amount of phosphorus that is absorbed. Since milk is a relatively phosphorus-rich food, there might be considerable impairment in its absorption without producing a depletion of the body's stores of this element. The relatively slight effect of magnesium trisilicate upon iron retention may be because there is relatively less magnesium present or because this element does not interfere with phosphorus absorption to a sufficient degree. The lack of effect of aluminum phosphate on iron retention might be due either to the fact that it cannot react further with phosphorus or because it tends to stabilize the reaction of the stomach contents at a lower pH (4). It is not pertinent to comment concerning the relative effect of these substances upon the reaction and secretion of the stomach and intestines.

From the data presented it is apparent that ingested iron is less efficiently utilized when the intake of calcium carbonate or aluminum hydroxide is high. To a lesser extent the same tendency is manifested by magnesium trisilicate. The impairment in iron retention could probably be overcome by the ingestion of additional iron. It appears reasonable to conclude that the iron intake of individuals ingesting relatively large quantities of these substances should be greater than for the average individual. This would be particularly important if accompanied by an excessive blood loss.

SUMMARY

It has been shown that calcium carbonate and aluminum hydroxide definitely reduce iron retention by the anemic rat ingesting 0.25 mgm. of iron daily. Magnesium trisilicate reduced iron retention somewhat but to a degree that is of questionable significance. Aluminum phosphate did not reduce iron retention. It is suggested that the iron intake needs to be greater in individuals consuming increased amounts of aluminum hydroxide or of calcium carbonate, than in the normal subject.

Acknowledgment. The authors wish to express appreciation for valuable advice given to them by Dr. F. C. Bing.

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THE PRODUCTION OF SHOCK BY TRAUMA AFTER SPINAL CORD TRANSECTION[1]

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Received for publication July 13, 1942

In previous experiments it was shown that a decrease in plasma volume resulting in shock could be produced experimentally by reducing the peripheral circulation. The decrease in blood flow resulted either from hemorrhage (1) or through injecting massive doses of adrenalin (2). It was further shown that when the extremities of dogs were traumatized under ether anesthesia a reduction in peripheral circulation preceded other signs of oncoming shock (3). Compression of the extremities by bandage and adhesive tape restricted the local fluid loss but did not prevent the reduction in blood volume as shock came on after trauma. Following recovery from total sympathectomy, trauma no longer caused a reduction in circulation but still a loss of blood volume was observed, greater than could be accounted for by the accumulation of fluid in the injured area (3). It was felt that the use of ether as an anesthetic might have influenced the results and accordingly the trauma experiments were repeated in dogs after recovery from spinal cord transection. Under these circumstances it was not necessary to use a general anesthetic.

METHODS. The spinal cord was transected between the first and second thoracic vertebrae in eight mongrel dogs. The animals were studied two or more days after recovery from this operation. The carotid artery and trachea were cannulated under local anesthesia. Oxygen consumption and arterial and venous oxygen and carbon dioxide concentrations were measured. The cardiac output was calculated by the Fick principle and blood volume determinations by the carbon monoxide method were made before and within three hours after trauma. Blood pressure was recorded from the carotid artery by means of a mercury manometer. Peripheral blood flow through an uninjured paw was measured by the plethysmograph. Hemoglobin and hematocrit determinations were made on blood obtained from an ear. The muscles of one hind leg were traumatized by 1,000 blows with a rubber hammer, according to the technic of Best and Solandt (4), and the bones of the extremity were fractured with a heavy metal bar. Blood loss into the area of injury was restricted by binding the extremity as suggested by Freedman and Kabat (5). The loss of blood into the area of injury was measured by the dissection technic of Cullen and Freeman (6) at the termination of the experiment. The visceral organs were examined after death.

[1] Aided by a grant from the National Research Council.

[2] Eli Lilly Fellow in Surgical Research.

[3] Fellow in the Harrison Department of Surgical Research.

RESULTS. Four control experiments were performed. One of these was discarded because of the poor condition of the animal due to wound infection and diarrhea. In the other three, as shown in table 1, there were no significant changes in the volume or character of the circulation during the experimental period. The course of a single control experiment is shown in figure 1.

In comparison with these results, after bone and muscle trauma a loss of blood volume was consistently observed. This loss of blood volume occurred within three hours after trauma while the circulation was still well maintained. Figure 2 shows the results obtained in the most striking experiment. The blood pressure and peripheral blood flow were not seriously reduced immediately after the trauma in three of the four experiments. A moderate reduction in cardiac

TABLE 1

DATE	EXPER. NO.	DOG NO.	WT.	DAYS AFTER TRANSECTION	BLOOD VOLUME			LOCAL FLUID LOSS	HEMOGLOBIN		HEMATOCRIT		BLOOD PRESSURE		BLOOD FLOW		CARDIAC OUTPUT		VENOUS OXYGEN SATURATION	
					Before trauma	After trauma	Difference		Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Control experiments																				
		kgm.			*cc.*	*cc.*	*cc.*	*gm.*	%	%	%	%							%	%
6-23	1	1026	14.2	6	1508	1346	162		82	84	30.5	34.5	140	105	2.2	2.4	8000*	3008	52.3	68.3
7-17	2	13	8.6	2	1166	991	175		91	91	18.6	18.5	94	96	6.0	4.0	1763	2035	66.0	80.0
7-10	3	1		2	1120	1118	2		93	95	35.2	35.2	110	96	10.7	11.3	1500	1319	59.6	59.6
7-14	4†	3	9.9	5	1931	928	1003		72	79	24.4	34.2	110	104	3.8	4.7	3015		62.7	
Trauma experiments																				
	5	2	11.8		2125	1029	1096	40	65	98	25.9	42.5	128	54	4.2	1.2	2520	737	60.7	45.2
7-24	6	7	9.0	16	1362	909	453	115	74	80	27.0	34.0	92	104	3.1	1.7	2170	882	68.9	41.0
6-27	7	1038	10.6	3	984	623	361	65	90	98	29.0	34.0	124	102	9.0	7.0	1226	868	50.0	47.2
6-25	8	1037		5	1600	1015	585	85	12.0 (O_2 capacity)	12.9	21.0	24.5	80	94	1.3	2.7	1993	1501	47.8	33.5

* Dog panting. Arterial oxygen saturation reduced.

† Experiment excluded because of the presence of wound infection and fever.

output was observed at the end of the experimental period in two of the dogs while it was marked in the other two.

DISCUSSION. It is agreed that tissue anoxia from reduced circulation causes capillary damage with consequent loss of plasma volume. The present experiments were designed to study the effect of trauma on blood volume where the injurious effects of reduced circulation were avoided. In addition, the possible complicating influence of a general anesthetic was eliminated by using dogs after spinal cord transection.

Reduced circulation was excluded in the present experiments by transecting the spinal cord between the first and second thoracic segments. By this procedure, not only were pain stimuli from the area of injury prevented, but also reflex efferent vasoconstrictor impulses were eliminated. These reflexes might have originated from unavoidable loss of blood or from fear and might have

caused splanchnic vasoconstriction. Reduced circulation from excessive local fluid loss also was avoided by binding the injured areas.

It is true that adequacy of the circulation to the viscera and to the muscles cannot be inferred from observations on the blood flow through the paw. The maintenance of cardiac output and oxygen saturation of mixed venous blood in three of the experiments, however, suggests that the general circulation was adequate during the experimental period. The moderate decrease in cardiac output in experiments three and four might well be attributed to the loss of blood volume. Repeated measurements of the peripheral circulation during the period after trauma, and before the second measurements of cardiac output and blood volume were made, suggested that the reduction in blood volume preceded the decrease in circulation.

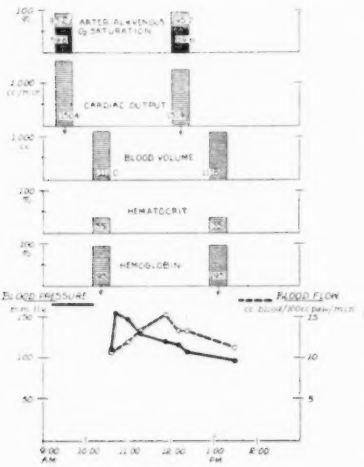

Fig. 1

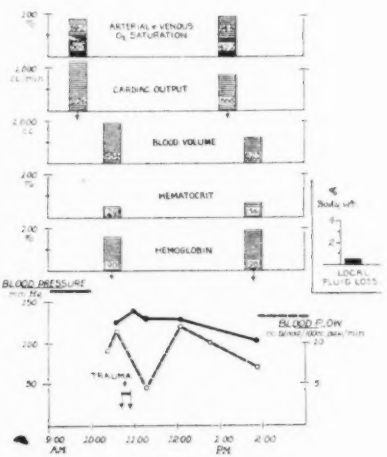

Fig. 2

Fig. 1. Effect of experimental procedures on the circulation of the dog after spinal cord transection, without trauma.

Fig. 2. Effect of trauma on the circulation of the dog after spinal cord transection.

In experiment 1 the blood pressure and blood flow were seriously depressed shortly after the trauma. It is possible that the reduction of blood volume in this experiment might have resulted from the circulatory defect. The possibility of fat embolism was considered as the cause of this fall in blood pressure but no fat droplets could be found in the pulmonary capillaries on microscopic examination of the lungs. That fat embolism could not have been etiologically significant in producing shock in the other experiments was shown by the fact that the blood pressure did not fall immediately after the trauma.

The effect of exclusion of sensory impulses upon the development of shock has been studied by various workers with different results. Simonart (7), O'Shaughnessy and Slome (8) and more recently Freeman and Kabat (5), believed that nervous impulses were of great significance, while Parsons and

Phemister (9), Freedlander and Lenhart (10), Holt and MacDonald (11), and Blalock (12) believed that nervous impulses were of secondary importance in the genesis of shock. It seems possible that the differences in opinion depend in part upon variations in the amount of blood and fluid unavoidably lost at the site of injury and upon the use of different criteria of shock. In experiments previously reported on the effects of trauma in sympathectomized dogs we judged, according to criteria of blood pressure, blood flow and even of recovery, that the process of shock had not been initiated. When we measured the blood volume, however, we found that it was reduced more than could be accounted for by the loss into the traumatized area. Shock is essentially the process of loss of blood volume due to increased capillary permeability. Only by adopting the criteria of reduced blood volume or increased capillary permeability is it possible to say whether or not the process has been initiated.

Objection may be raised that the most striking experiment with trauma was selected for comparison with a control experiment. This selection was made since in this case, above all, a significant reduction in blood volume occurred in spite of a circulation which was apparently adequately maintained. The evidence in the remaining experiments supported the concept to which we had been led. Furthermore, the present experiments confirm the observations which were made upon the reduction of blood volume after trauma in sympathectomized dogs. Under those circumstances reduced circulation was again not a significant factor.

Post mortem examination disclosed congestion of the mucosa of the upper intestinal tract with edema of the intestinal wall and even some free fluid in the lumen of the gut. These findings are characteristic of shock. Exactly by what mechanism this loss of blood volume occurred is obscure. It was present, although less pronounced, even in the two dogs which were sacrificed while their circulation was still adequate. When reduced circulation persisted for an hour or more, the course of shock appeared to be hastened and the pathological changes in the viscera were more intense.

SUMMARY AND CONCLUSIONS

1. Bone and muscle trauma was inflicted upon four dogs after recovery from spinal cord transection.

2. Four control experiments without trauma were performed.

3. The adequacy of circulation was determined by measurements of blood pressure, peripheral blood flow, cardiac output and oxygen content of mixed venous blood.

4. The amount of fluid loss into the traumatized area was measured and was kept at a low level by binding the injured extremity.

5. A reduction of blood volume and hemoconcentration occurred after trauma in the presence of a well-maintained circulation and in the absence of excessive blood loss into the injured extremities.

6. Post-mortem examinations revealed pathological changes characteristic of shock.

7. These findings suggest the action of some factor capable of causing a reduction in blood volume not primarily due to excessive local fluid loss or to reduced circulation.

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NITROGEN CLEARANCE FROM THE BLOOD AND SALIVA BY OXYGEN BREATHING

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Received for publication July 15, 1942

Several investigators have shown that the course of the elimination of nitrogen from the body during the breathing of pure oxygen follows an exponential curve (Campbell and Hill, 1931, 1933; Behnke, Thomson and Shaw, 1935; Behnke and Willmon, 1941). The latter authors found that 40 to 50 per cent of the total nitrogen was eliminated during the course of the first hour, whereas 80 to 90 per cent clearance required 4 to 5 hours. No direct determinations of the nitrogen content of blood and saliva during clearance seem to have been reported in the literature. The present paper describes the nitrogen clearance from human blood and saliva during a period of breathing pure tank oxygen, as determined by a micro-gasometric technique. Small samples allowed frequent determinations both conveniently and accurately.

TECHNIQUE. The measurements of the nitrogen content of the blood and saliva were made by means of a method for the determination of nitrogen in small amounts of blood and saliva and other fluids (Scholander, 1942). Samples of 40 cu. mm. of saliva were obtained anaerobically from the ducts of the sublingual glands. Each subject was in a resting position during the experiment, and was provided with a nose mask through which he could breathe pure tank oxygen. Samples of blood and saliva were taken and analysed at frequent intervals before, during, and after the period of oxygen breathing.

RESULTS. The figure shows three curves of the nitrogen clearance from the finger blood and two curves of the nitrogen clearance from the saliva. Four different subjects were used. The samples of normal blood and saliva contained an average of 1.02 volume per cent nitrogen, with a range from 0.97 to 1.05 volume per cent. The curves show that the nitrogen was eliminated rapidly from both finger blood and saliva during the period of oxygen breathing. Eighty to 90 per cent of the nitrogen was cleared within the first 10 minutes. The remaining 10 to 20 per cent was gradually eliminated during the next 50 minutes, after which time the oxygen mask was removed. At the end of 1 hour the nitrogen content of the blood and saliva of the subjects had reached the low value of 0.06 volume per cent. Beyond this point the analytical procedure and sampling were less certain on account of the smallness of the bubble to be measured and the danger of air contamination of the samples. When the subjects breathed air again after the removal of the mask, the nitrogen in the blood and saliva usually showed a transitory rise above normal. This excess in recovery was also noticed in preliminary experiments, but the reason for its occurrence is undetermined. The rate of resaturation of the blood and saliva with nitrogen

from the air was practically as rapid as the rate of desaturation during the oxygen breathing, and both processes occurred within 10 minutes. The clearance figures for finger blood nitrogen and saliva nitrogen did not show any significant differences.

The experiments show that the nitrogen is cleared rapidly from both finger blood and saliva as compared with the very slow total clearance, as determined by Behnke and Willmon (1941). The clearance curves for the finger blood and for the saliva are practically identical and are probably close to the clearance

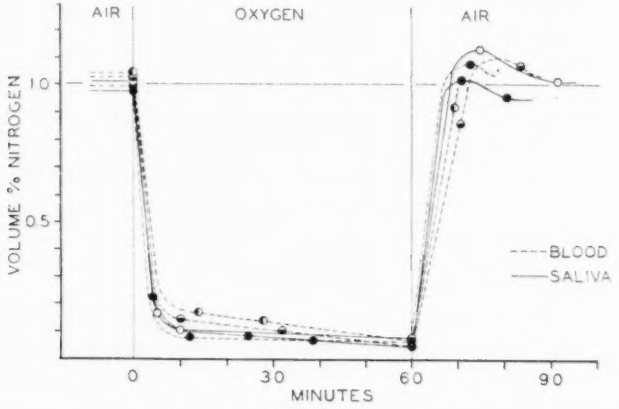

Fig. 1. Curves of the elimination of nitrogen from the finger blood and the saliva of four different persons at rest. The subjects are represented by the various types of circles.

curves of arterial blood. They are probably more closely indicative of the alveolar nitrogen than of the tissue nitrogen.

We wish to express our appreciation to Dr. Laurence Irving for his help and support.

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MECHANISM OF THE EFFECT OF EPINEPHRINE ON THE VENOUS HEMATOCRIT VALUE OF THE NORMAL UNANESTHETISED DOG

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Received for publication July 16, 1942

We submit evidence below to show that hemoconcentration as indicated by an increase in the venous hematocrit reading does not necessarily represent a change in the mass of the circulating red blood cells. By the use of red cells tagged with the radioactive isotope of iron[2] we have found that there are no large reserve stores of red cells in the dog but that essentially all of the cells are in active circulation (5). Stead and Ebert by measurement of the hemoglobin removed by phlebotomy and comparison with the changes in circulating hemoglobin as determined indirectly with plasma volume estimations arrived at the conclusion that such was also the case in human subjects (8). The data presented herein are in accord with these findings and suggest that a rearrangement of the distribution of vascular components must be considered in obtaining an adequate conception of the status of the circulatory system at a given time.

That the intravenous administration of epinephrine sometimes results in an increase in the peripheral red cell count and the venous hematocrit value was pointed out in 1915 by Lamson (7), who felt that the change was due to liberation of cells stored by the liver. Barcroft and his associates (1) (2) did not subscribe to the idea that the liver acted as an important storehouse for red cells but stated that the spleen served in this capacity. Furthermore Barcroft (2) as well as Cannon and Izquierdo (3) found that although the administration of adrenalin did not invariably increase the peripheral red count, it did cause contracture of the spleen. The net result of these observations has been the generally accepted assumption that when the drug epinephrine is given by vein the resultant apparent increase of the red cells in the circulation is due to liberation of cells due to splenic contracture.

With a means of differentiating between red cells in active circulation and those which might be temporarily sequestered by splanchnic or other storage, the above concept should readily lend itself to test. Circulating red cells may be tagged effectively by the addition of other cells to the circulation, the latter having been formed in a donor animal of the same species, the hemoglobin of which has some of its constituent iron in the form of the radioactive isotope (5) (6). If the total radioactivity of the injected cells is known it becomes pos-

[1] We are indebted to the Eli Lilly Company for aid in conducting this work.

[2] We wish to express appreciation to the Radiation Laboratory at Berkeley, California, and in particular to Drs. E. O. Lawrence and M. D. Kamen for the radioactive iron used in these experiments.

sible to measure the circulating mass of red cells of the recipient after allowing sufficient time for mixing of the cells. If the spleen holds an appreciable number of red cells from the active circulation, administration of adrenalin, by contraction of this organ, should result in a further measurable dilution of the circulating red cell radioactivity. A clear cut demonstration would necessarily be dependent on a minimal rate of circulation of red cells in the spleen during the period of cell mass estimation.

METHODS. Duplicate blood samples were drawn from the jugular veins of adult dogs. Twenty-five milliliters of whole citrated or heparinised blood containing a known amount of red cell radioactivity were injected into the same vein in a period of about fifteen seconds. After time for complete mixing (6), usually four or five minutes, 25 ml. of blood were withdrawn from the same vein into 5 ml. of isotonic (1.4 per cent) sodium oxalate. One-half milliliter of epinephrine (1:1,000) was then injected through the same needle and after three minutes another 25 ml. of blood were taken. In some instances another injection of donor blood was carried out with subsequent sampling after four or five minutes. There was no stasis at any time except during the initial venepuncture. The needle was allowed to remain in the vein throughout the experiment and was occasionally flushed with blood from the circulation by alternately withdrawing and reinjecting a few milliliters of blood with a syringe. Sampled blood was divided into three parts for triplicate determination of radioactivity content and hematocrit measurement. Blood was centrifuged for thirty-five minutes at about 2500 r.p.m. Ashing of the red cells, separation and electroplating of the contained iron, and determination of the radioactivity were carried out as described elsewhere (4).

Radioactive iron was prepared by the method of Wilson and Kamen (9).

EXPERIMENTAL OBSERVATIONS. A preliminary series of injections of epinephrine in the same dosage as used in these experiments showed that under these conditions the elevation of venous hematrocrit reading was rather a transient phenomenon. The maximum level was reached in the neighborhood of one minute and the hematocrit usually had reached its initial level in five minutes. Cell mass was not measured after one minute however since it would not allow time for complete mixing. Therefore a compromise was made on a three minute sampling, but it must be kept in mind that this might well have affected the degree of responses shown in tables 1 and 2.

Under these conditions the increase can be seen not to have been an invariable effect obtained following epinephrine injection. In general the response was consistent and of about the same degree in the same dog. Some animals repeatedly showed minimal changes and obviously were of no interest for the study of relationship between cell mass and hematocrit changes.

In table 1 it is to be noted that even in the animals in which a fairly uniform increase in hematocrit follows adrenalin injection, when the animals were splenectomised the response was abolished. In fact in no animal studied in which splenectomy had been performed have we observed such a response. Except for the evidence in table 2, in which there is found no relationship between the

red cell mass as directly determined and the venous hematocrit changes, such a finding would be construed as further evidence that the splenic contraction following adrenalin normally liberated red cells to the active circulation causing an increased hematocrit value. We are not prepared to say on a basis of the evidence available what the correct explanation may be. It is conceivable that the spleen acts in some altogether different fashion in conjunction with epinephrine to affect the distribution of the vascular constituents. An assumption that the circulation rate through the spleen is of the same order of magnitude as that of the general circulation, and that the effect of adrenalin is to cause a preferential loss of red cells from the splenic pulp while retaining a considerable amount of plasma might explain the findings, but there is no evidence to support this here.

It should also be pointed out that the adrenalin response does not seem to be related to the degree of anemia in these dogs, although unexpectedly the greatest hematocrit value changes occurred in the extremely anemic animals, 39–196 and 39–299, table 1. From a viewpoint of splenic storage it would be difficult to conceive of an animal with a severe anemia having a reserve of red cells withheld from the active circulation.

Discussion. We may consider in what the mechanism of increased hematocrit values following adrenalin may consist. Since the red cells of the dog are all in active circulation in the normal and anemic animal (5) (6) the various fractions of the vascular fluids which we must consider are then: the red cell mass (CV), the volume of plasma in rapid circulation (PV_c), and the volume of plasma in sluggish circulation (PV_s). The normal partition of these fractions has been discussed in some detail elsewhere (6). The changes in the distribution of these constituents in which we might find an increased venous hematocrit reading are then:

1. An increase in CV with no change in PV_c .
2. An increase in CV with an accompanying smaller increase in PV_c .
3. A decrease in CV with a greater decrease in PV_c .
4. No change in CV with an accompanying decrease in PV_c .

Since with the administration of adrenalin it is shown that CV does not change greatly we may assume that condition four above best fits the findings under these circumstances. This would, in view of the rather rapid resumption of the hematocrit level to its former value after adrenalin injection, indicate a temporary movement of some of the PV_c fraction to the PV_s . It seems doubtful if this decrease in rapidly circulating plasma takes place through loss of fluid from the vascular system. If it does it should be accompanied by an increased plasma protein concentration which was unfortunately not followed in these experiments. However the transient nature of the reaction argues against the quite sudden loss and gain of vascular fluid within a period of five minutes or less.

If the effective number of blood vessels of small inside diameter were markedly increased, or if by vasoconstriction the caliber of a large number of vessels were decreased, the effect would be to increase the amount of plasma in sluggish circulation (6). This would seem to be a more reasonable explanation of the

TABLE 1

Venous hematocrit changes following the injection of epinephrine by vein

DOG	RED CELL HEMATOCRIT			DOG	RED CELL HEMATOCRIT		
	Initial	After adrenalin	Rise		Initial	After adrenalin	Rise
	per cent	*per cent*	*per cent*		*per cent*	*per cent*	*per cent*
40-183	26.8	36.1	29.8	40-115 splenect.	31.8	31.5	−0.9
40-183	38.3	49.2	28.4	40-115 splenect.	45.7	45.1	−1.3
40-183	48.4	55.6	14.9				
				40-194 splenect.	38.9	40.0	2.8
30-299	42.4	49.8	17.5				
30-299	36.0	40.9	13.6	36-196	13.6	19.6	44.2
30-299	11.9	16.4	37.8	36-196	23.0	22.6	−1.7
				36-196	26.3	34.0	29.2
41-455	53.4	55.5	3.9	36-196 splenect.	29.5	31.5	6.8
				36-196 splenect.	32.9	34.6	5.2
41-557	43.8	45.2	3.2				
				1-G	41.4	49.8	20.2
40-15	30.8	36.2	17.5	1-G splenect.	36.7	37.4	1.9
				1-G splenect.	38.1	38.5	1.1
4-E	45.7	47.2	3.3				
4-E	42.2	42.7	1.2	2-G	46.1	48.4	5.0
4-E	23.3	23.9	2.6	2-G splenect.	28.2	29.1	3.2
4-E	22.5	22.3	−0.9	2-G splenect.	30.4	32.2	5.9
4-E	12.0	12.5	4.2				
39-57	56.0	59.8	6.8				
39-320	23.2	29.6	27.6				
39-320	30.3	35.6	17.5				
39-320	44.5	51.9	16.6				
39-320	48.8	58.8	20.5				
39-320 splenect.	36.8	36.5	−0.8				
39-320 splenect.	38.1	38.4	0.8				

TABLE 2

Relative constancy of red cell mass with increased venous hematocrit following adrenalin

DOG	RED CELL HEMATOCRIT			RED CELL CIRCULATING MASS		
	Initial	After adrenalin	Rise	Initial	After adrenalin	Rise
	per cent	*per cent*	*per cent*	*ml.*	*ml.*	*per cent*
39-320	44.5	51.9	16.6	347	347	0
39-299	36.0	40.9	13.6	366	369	0.8
39-196	26.3	34.0	29.2	234	244	4.3
39-196	13.6	19.6	44.2	125	129	3.2
39-196 splenect.	32.9	34.6	5.2	321	340	6.2
1-G	41.4	49.8	20.2	470	490	4.3
1-G splenect.	36.7	37.4	1.9	518	542	4.6

reaction especially when the nature of the pharmacological action of epinephrine is recalled. We should not lose sight of the possible change in vascular set-up which results from partial constriction of vessels such that most of the red cells in the axial stream are washed out leaving what amounts to tubes of cell free plasma. Such blood vessels are probably contracted to somewhat less that 7 microns and would contribute greatly to the fraction of plasma in sluggish circulation.

If there is a movement of plasma from the rapidly circulating to the sluggish moving state as a result of adrenalin administration the effect is similar to that which would be obtained by the removal of plasma from the active circulation and therefore the use of adrenalin in certain types of shock might be contra-indicated.

Reasoning teleologically we might say that such a shift in the rapidly moving plasma fraction resulting in a hematocrit reading increase might constitute a useful mechanism whereby in an emergency when epinephrine is poured into the circulation by physiological response, an increase in gas exchange is facilitated. The reaction being quite transitory the distribution of the plasma and red cells is then quickly restored to a normal state.

SUMMARY

When epinephrine is administered by vein in single large doses to adult intact dogs there is sometimes a decided increase in venous hematocrit readings. Some dogs uniformly show a marked increase whereas others rather consistently show minimal changes.

In the animals in which there is an increase in venous hematocrit reading there is no corresponding change in the circulating red blood cell mass as directly determined by means of the donor-isotope-red cell procedure.

Since splenectomy abolishes the hematocrit response to epinephrine and since no reserve cells are found to be poured into the circulation following adrenalin, another mechanism than outpouring of reserve red cells from the contracted spleen must be postulated to account for the increase in venous hematocrit value following the administration of this drug.

A suggested explanation of the phenomenon may be an increase in the effective number of blood vessels of small inside diameter as well as a reduction in the caliber of many vessels by vasoconstriction, both factors leading to an increased fraction of plasma in sluggish circulation.

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THE DISTRIBUTION OF SUCROSE IN BODY FLUIDS FOLLOWING INTRAVENOUS INJECTIONS

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Received for publication July 17, 1942

In recent years the osmotic effects of hypertonic sucrose solutions have been widely utilized in a variety of conditions, particularly for the reduction of increased intracranial pressure and for the production of diuresis.

Articles on the clinical use of sucrose repeatedly state that sucrose possesses an advantage over other sugars such as glucose because it does not readily pass through capillary walls, does not enter most body fluids and is not absorbed by body tissues. This supposed non-diffusibility of sucrose is frequently contrasted to the rapid diffusibility of glucose and saline solutions. The experimental evidence does not support these conclusions. Keith, Power and Peterson (1) found that in dogs, a large percentage of the injected sucrose disappeared rapidly from the blood stream and could be detected in the tissues. Lavietes, Bourdillon and Klinghoffer (2) found that sucrose was distributed in approximately the same fraction of the body fluids as thiocyanate and inorganic sulfate, and could be used as a measure of extracellular fluid volume. Hubbard and Terplan (3) were able to detect sucrose in the spinal fluid following intravenous injection. In a preliminary report we presented evidence that sucrose entered readily into certain other body fluids (4).

We have attempted to clarify the confusion regarding the diffusibility of sucrose by studying its distribution in patients with abnormal accumulations of extracellular fluids and by comparing their rate of eliminating sucrose from the blood with that of a control group.

This study has been considerably facilitated by the use of a differential fermentation method for sucrose which has enabled us to measure very small amounts of sucrose with considerable accuracy.

METHODS. Our control group consisted of ten patients admitted to this hospital for a variety of conditions but without evidence of renal or circulatory failure and without edema or other abnormal fluid accumulations. The second group included nine patients with anasarca, edema, ascites or pleural effusions from a variety of causes as shown in table 3.

Each patient received an intravenous injection of 60 cc. of 50 per cent sucrose in a period of approximately five minutes. Reactions were few and seldom alarming. Sterile blood samples were taken at the intervals shown in the tables. Sterile body fluids were collected by paracentesis, thoracentesis or by the use of large needles inserted subcutaneously in edematous cases.

Sucrose was determined by the resorcinol method of Roe (5) after differential fermentation using cultures of B. coli communis (Escherichia coli) and B. coli

communior (Escherichia communis). The latter will ferment glucose, sucrose and other sugars while the former will ferment glucose and other sugars but not sucrose. The difference between the two samples, therefore, represents the sucrose concentration. Sterile samples (usually 2 cc.) of serum or fluid were heavily inoculated with cultures of B. coli communis and communior respectively and incubated twenty-four to forty-eight hours. Previous to inoculation, all samples were cultured for sterility and any found contaminated were discarded. Following incubation a protein free filtrate was prepared by the zinc method of Somogyi (6) using a dilution of 1:10 for the serum and 1:5 for other body fluids. The sucrose concentration in the filtrate was then determined by the resorcinol method of Roe for fructose, using a series of sucrose standards of varying dilution. Those above 0.5 mgm. per cent were read in a colorimeter. Lower values were estimated by direct comparison.

The blank in the communior sample (from which all sugars had been removed by bacterial action) was subtracted from the color in the communis sample. This blank almost invariably gave a color less than that equivalent 0.5 mgm. per cent of sucrose.

TABLE 1

Recovery of sucrose added to serum and to ascitic fluid

BLOOD SERUM		ASCITIC FLUID	
Calculated	Determined	Calculated	Determined
84	92	36	37
42	42	11	12
17	16	2	1
2	2		

We found that the values obtained by the Roe method were similar when samples were hydrolyzed and compared with fructose standards and when compared with sucrose standards without hydrolysis. Therefore, sucrose standards were used and hydrolysis omitted throughout this study.

Identical colors were obtained with and without centrifuging after inoculation to remove suspended bacteria, showing that the presence of the organisms themselves did not affect color production. Cultures of B. coli communior were added to glucose and sucrose containing sera and identical blanks obtained, indicating that any difference in the fermentation of these sugars did not influence color development. Identical blanks were also obtained when each organism was added to glucose-containing sera indicating that any differences in the metabolism of these two strains of the organism did not influence the determination.

We were able to obtain good recovery of sucrose added to serum and to ascitic fluid as shown in table 1.

RESULTS. 1. *Study of the rate of disappearance of sucrose from the blood stream in patients without evidence of renal or circulatory failure and without abnormal*

fluid accumulations. Following the injection of sucrose, blood was taken at varying intervals in different individuals. The results in the ten patients have been combined in table 2. It will be seen that following an initial high level, there is an early rapid drop followed by a more gradual fall, only traces being present after twenty-four hours, the serum containing no sucrose after forty-eight hours. These findings are in accordance with those of Keith and Power who were able to recover eighty-nine to ninety-eight per cent of injected sucrose within twelve to twenty-four hours after injection (7).

2. *Study of the distribution of sucrose in patients with abnormal extracellular fluid accumulations.* The results in patients with abnormal fluid accumulations are in marked contrast to the controls. An examination of table 3 reveals that sucrose enters the body fluids studied very readily. In the case of edema fluid, considerable concentrations were present as early as ten to fifteen minutes following the injection. It remained within these body fluids for considerable

TABLE 2

Rate of disappearance of sucrose from the blood stream in the control group following intravenous injection

INTERVAL FOLLOWING INJECTION		BLOOD SERUM LEVEL	INTERVAL FOLLOWING INJECTION		BLOOD SERUM LEVEL
Hours	Minutes	Mgm. per cent	Hours	Minutes	Mgm. per cent
	2	320	18		4
	5	312	18		1
	5	258	21		2
	15	200	20		1.5
	35	228	24		0.7
2	45	104	24		1.5
4	50	45	24		1.5
6	15	44	48		none
8		17	48		none
11		9			

periods of time, these fluids apparently serving as a reservoir for sucrose and maintaining a relatively high level in the blood for much longer periods than in normal individuals. Initially the sucrose in the blood is much higher than in the body fluid, followed by a period of approximately equal concentration, the blood level finally tending to fall below that of the fluid.

Discussion. Contrary to many statements in the literature, our results indicate that sucrose diffuses rapidly into body fluids. We realize it is possible that in cases with abnormal fluid accumulations, the capillaries may have greater than normal permeability. Nevertheless, it is in just such patients that sucrose is often used clinically as a diuretic and hence any supposed advantage in its use in such instances cannot be based on lack of tissue penetration. To us, it seems unlikely that sucrose would fail to penetrate into extracellular fluids even in normal individuals since sucrose is a relatively small, non-electrolyte molecule. As previously stated, there is experimental evidence in the literature to support its diffusibility into extracellular fluids.

In our experiments, we did not attempt to evaluate the clinical advantages of sucrose as compared with other sugars. The amounts of sucrose we injected

TABLE 3

Distribution of sucrose in patients with abnormal accumulations of extracellular fluids

NO.	CLINICAL DIAGNOSIS	FLUIDS STUDIED	INTERVAL AFTER INJECTION		BLOOD SERUM CONCENTRATION	CONCENTRATION IN FLUID
			Hours	Minutes	Mgm. per cent	Mgm. per cent
1	Cirrhosis of liver	Abdominal	1½		211	72
			24		52	60
			51		20	27
			97		9	9
2	Cardiac failure, hydrothorax	Pleural	24		8	25
3	Cirrhosis of liver	Abdominal	20		2	8
4	Cardiac failure, hydrothorax	Pleural	3		7	35
5	Pleurisy with effusion (tuberculous)	Pleural	24		1	4
6	Cirrhosis of liver	Abdominal	23		6	25
7	Cirrhosis of liver, hypoproteinemia	Edema fluid		35		12
			1			18
			2			31
			3½			36
			5			36
			7			38
			24		8	10
8	Cirrhosis of liver, hypoproteinemia	Edema fluid		15		16
				45		31
			1¼			44
			1¾			55
			2¼			59
			2¾			59
			3¼			48
			4¼		31	
		Abdominal	24		2	5
9	Chronic nephritis with edema	Edema fluid		10		12
				25		19
				45		24
			1			31
			1	20		33
			1	45		29

are smaller than those usually employed for therapeutic effect and no attempt was made to estimate the amount of diuresis, fall in intracranial pressure or

other clinical effect. Our results should not be interpreted to contradict the many articles in the literature wherein the authors claim a superiority for hypertonic sucrose solutions as compared with those of glucose. However, we do believe our experiments indicate that claims to such superiority should not be based upon lack of diffusibility into extracellular fluid.

SUMMARY

1. An accurate method for the determination of sucrose is presented. Sterile samples are incubated with cultures of Bacillus coli communior and Bacillus coli communis and sucrose determined by appropriate modification of Roe's method for fructose. Since the former organism ferments sucrose while the latter does not, the difference in the two samples represents sucrose.

2. Sucrose was injected intravenously into patients who had no evidence of renal or circulatory failure and without abnormal fluid accumulations, and its rate of disappearance from the blood stream was studied. Only traces were present after twenty-four hours and the serum contained no sucrose after forty-eight hours.

3. In patients with abnormal extracellular fluid accumulations, sucrose was found to diffuse rapidly into ascitic, pleural and edema fluids, following intravenous injection. These fluids were found to act as reservoirs for sucrose, so that it was retained in the tissues and remained at relatively high levels in the blood for long periods of time.

The authors wish to express their gratitude to Mrs. Elfriede Fendt Sicari for her technical assistance in the bacteriologic phases of this study.

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THE INFLUENCE OF THE ACCELERATOR NERVES ON THE BASAL HEART RATE OF THE DOG[1]

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Received for publication July 20, 1942

Most investigators have believed that the cardio-accelerator fibers are in a state of tonic activity. Hunt (1) working with anesthetized dogs found that section of the accelerator nerves to the heart seldom failed to cause slowing. In experiments cited on three dogs, the heart rates before cutting the accelerator nerves were 237, 219 and 174 beats per minute. After cutting the accelerator nerves, these heart rates fell respectively to 159, 138 and 159 beats per minute. Hunt noted this decrease in the heart rate following section of the cardio-accelerator fibers regardless of the drug used as the anesthetic agent and when anesthesia was produced by section of the crura cerebri or by compression of the cerebrum.

Bronk and his collaborators (2, 3, 4) studied the activity of the cardiac sympathetic center by recording the action potentials in the cardiac-nerves from the stellate ganglion of the cat. He found a continuous discharge of impulses from these accelerator nerves which he interpreted as evidence for tonic activity of the accelerator nerves.

Gasser and Meek (5) studied the effect of removal of the stellate ganglia on the heart rate of trained dogs at rest. In this instance the normal pulse rates of the dogs at rest were determined by making them lie quietly until the pulse rates no longer decreased. The stellate ganglia were removed and after the dogs recovered their resting heart rates were again determined. The resting heart rates of the six dogs reported ranged from 78 to 140 beats per minute before removal of the stellates. After the removal of the stellates, the range of the resting heart rates was from 66 to 80 beats per minutes. This decrease in the resting heart rate after the removal of the stellates suggested accelerator tone.

In the present work dogs were trained until their resting heart rates reached a value which was low and consistent on consecutive determinations and which was considered the basal resting rate. The effect of cardiac sympathectomy on this basal rate was then investigated.

METHODS AND RESULTS. To establish the basal heart rates normal dogs were trained by gentle methods to lie quietly on a table for a period of 60 minutes each day. The dogs were not allowed to eat any food during the 12 hour period preceding each rest period. Extraneous noises were reduced to a minimum and the apprehension of the dogs was also reduced as much as possible. The animals did not go to sleep while on the table. Pulse rates were taken frequently by palpation from the femoral artery until the dogs became accustomed to the procedure. Some of the dogs were accustomed to the electrocardiograph elec-

[1] Supported in part by a grant from the Wisconsin Alumni Research Foundation.

trodes and to the noise made by the machine so that heart rates could be obtained by watching the beam of the electrocardiograph and electrocardiograms could be taken to verify the heart rates obtained by palpation. Since sinus arrhythmia frequently accompanied slow heart rates, the pulse was counted for 60 seconds each time the heart rate was determined.

Resting heart rates were obtained on 24 dogs. In table 1 it can be seen that the average heart rate before conditioning the dogs was 96 beats per minute.

TABLE 1

Resting heart rates of dogs at near basal conditions and the effect of cardiac sympathectomy on such rates

DOG NO.	HEART RATE BEFORE CONDITIONING	USUAL RANGE OF HEART RATE AT NEAR BASAL CONDITIONS		TIME OF LAST OBSERVATION AFTER SYMPATHECTOMY	HEART RATE OF LAST OBSERVATION AFTER SYMPATHECTOMY
		Before sympathectomy	After sympathectomy		
	beats/min.	*beats/min.*	*beats/min.*	*days*	*beats/min.*
1	100	56–65	60–65	70	72
2	95	42–50	42–50	24	45
3	95	42–55	42–55	70	48
4	95	45–55	48–55	34	48
5	90	44–50	54–60	55	60
6	110	55–65	60–65	21	64
7	96	46–65	54–65	21	58
8	90	43–55	42–55	35	55
9	112	52–60	48–60	12	58
10	96	44–50	48–50	11	48
11	95	57–65			
12	80	55–60			
13	85	55–60			
14	90	44–55			
15	95	50–55			
16	95	44–50			
17	130	44–50			
18	120	45–50			
19	85	55–60			
20	90	45–50			
21	96	45–50			
22	85	50–60			
23	80	48–55			
24	90	50–60			
Average.....	96	50–56			

This rate was obtained on the first day of conditioning after the animal had lain quietly for at least 10 minutes. Also recorded in table 1 is the usual range of the heart rates of each of the dogs at near basal conditions. The lowest rate in this range was obtained in some dogs after a very few days and on several occasions. However, with other dogs a period of several weeks of conditioning was necessary before such a rate could be obtained, and even then it might be reached only once during the period of conditioning. After the basal heart rate was reached it seldom varied on consecutive determinations by more than 10 beats

per minute by the end of the 60 minute rest period. Twelve of the twenty-four dogs had a resting rate of 45 beats per minute or less on at least one day while the basal rates were being determined, and no rate failed to slow below 58 beats per minute during the period of conditioning.

In 10 of the trained dogs the stellate ganglia and sympathetic chains with the upper five thoracic ganglia were removed. The surgical procedure was completed in a single stage in all of the dogs except dog 3. In this animal a two stage removal was performed with the second stage following the first by a week.

After the dogs recovered the resting heart rates were again observed. The resting rates on the first few days following the sympathectomy were considerably above the basal level, but only the dog from which the sympathetics were removed in two stages failed to return within 14 days approximately to the heart rates before sympathectomy recorded in table 1. Six of the dogs returned to the normal basal rate within 7 days. In dog 5 there was an increase of 10 beats per minute in the resting heart rate after the sympathectomy Dog 7 showed an increase of 8 beats per minute after the sympathectomy. In the remaining dogs there was only a difference of 5 beats per minute or less from that established as the basal resting rate before the operation. Thus no significant difference between the basal heart rates before and after the removal of the accelerator nerves was found although 8 of the dogs were tested for 21 days or more following the sympathectomy.

This is evidence opposed to the general idea that the accelerators are in a constant state of tonic activity.

Discussion. Since the present data show that the heart rate of the normal dog at rest may be brought into a range of 42 to 65 beats per minute, the results of Hunt should not be indicative of accelerator tonus in a normal resting dog. Under the conditions of his experiment there is no doubt that the accelerator center in his dogs was in a state of activity, in some cases almost maximum activity, as evidenced by the very high heart rates he obtained before the cardiac sympathetics were cut. Even after cutting the cardiac sympathetics the heart rates Hunt obtained were considerably above those found for the normal resting, unanesthetized dog as given in the present report.

Gasser and Meek did not bring their dogs to a basal level, meaning the lowest rate that could be attained by training. Undoubtedly, however, the care subsequent to the operation brought their animals somewhat nearer such a level, as is indicated by the uniformity in the pulse rates after the removal of the stellate ganglia. If a true resting rate was not attained before the operation, any approach to it after the removal of the stellates would naturally have been attributed by these authors as evidence of the loss of accelerator tone.

Bronk and his collaborators (2, 3, 4) have shown that in nembutalized cats there is a more or less constant flow of impulses out over the cardiac sympathetics. However, these impulses were reduced or abolished by conditions which increase vagal tone such as adrenalin injection or increased pressure in the carotid sinus. This would indicate that as the cardio-inhibitory center was stimulated the accelerator center was reciprocally inhibited.

These results of Bronk's are not inconsistent with the present observations.

Under ordinary conditions the heart is partly held at a higher rate by reduced vagal tone. Evidence for this may be found in the results of atropine injection which at once raises the basal resting rate, and in the motility of the heart rate in exercised animals deprived of their sympathetics as shown by Gasser and Meek.

In view of past work and the present data it would seem that the accelerators in the intact animal are concerned with adapting the heart rate to any of the constantly occurring changes in bodily conditions. During the ordinary waking hours both the vagal and accelerator centers are being constantly bombarded by impulses from various sense organs and psychical regions of the brain. Thus the heart rate is constantly maintained above its possible basal rate both because of reduced vagal tone and increased accelerator tone. However, when the animal is shielded from these effects of sensation and emotion to a considerable extent as is done when a basal heart rate is reached, the accelerator mechanism ceases its activity and the heart rate slows to a rate determined largely or entirely by vagal tone. For this basal rate it does not matter whether the accelerator mechanism is in existence or not. The accelerator mechanism would therefore seem to be truly an emergency mechanism.

If this conception holds it would be the function of the vagus to determine a true basal resting rate. In a trained animal lying comfortably in a noiseless room there would be nothing to throw the accelerator mechanism into activity either by direct sensory impulses or by a reflex secretion of adrenalin sufficient to affect the heart directly. In this resting condition the accelerators do not seem to be in tonic activity, otherwise the basal resting rate before and after cardiac sympathectomy could not be identical.

SUMMARY

The near basal heart rates of normal unapprehensive dogs that had been without food for 12 hours and had rested quietly for 60 minutes ranged from 50 to 56 beats per minute.

Bilateral removal of the stellate and upper 5 thoracic ganglia failed to result in an appreciable change in this basal heart rate.

The author wishes to thank Dr. W. J. Meek and Dr. C. R. Allen for advice and assistance in this work.

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THE EFFECTS OF A DIET DEFICIENT IN THE VITAMIN B COMPLEX ON SEDENTARY MEN

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Received for publication July 9, 1942

In a general way, we know the subsistence requirements of the essential foodstuffs, but it is uncertain what amounts are necessary for the highest efficiency, and little is known about what effects the sudden withdrawal of any of the dietary essentials may have upon a man's capacity to carry on his usual work. The present experiments are a contribution to one aspect of this problem. Sedentary subjects lived on a diet grossly deficient in the vitamin B complex. The course of the deficiency was followed by the amount of thiamine excreted in the urine, since this reflects closely and rapidly the dietary intake of thiamine (23). In addition most students of experimental B deficiencies have stressed the constant, early appearance of easy fatigue as a symptom (15, 29). Therefore, particular attention was paid to the reactions of our subjects to easy work in the shape of walking uphill and to exhausting work in the shape of running uphill on a motor-driven treadmill.

Our results show clearly that under the dietary regime described below, all subjects become deficient at least in thiamine within three or four weeks, that there is measurable physical deterioration in this time, and that brewers' yeast is a complete and adequate supplement to the deficient diet that we used.

EXPERIMENTAL PROCEDURE. For clarity in the rest of the paper the various periods in the experiment on each subject will be called, in order, the *normal period before*, lasting about one week, in which the subject ate his usual diet; the *deficient period*, lasting from three to four weeks, during which he ate the diet deficient in the vitamin B complex; the *yeast period*, lasting one or two weeks, in which he continued the deficient diet, but took brewers' yeast every day; and the *normal period after*, in which he reverted to his usual diet. The periods followed each other without interruption, and the subject carried on his usual laboratory and hospital duties all the time. The measurements described in detail below were made in each period.

The seven subjects were healthy physicians between 27 and 42 years of age. None was a trained athlete, and all were leading the sedentary life customary among laboratory workers. They were allowed unlimited amounts of unfortified white bread, soda crackers, butter, cheddar type cheese, macaroni, spaghetti, polished rice, farina, heavy cream, sugar, honey, molasses, tapioca, salad oil, egg white, ice cream, puffed rice, coffee, tea, salt, pepper, vinegar, hard candies, and gelatine. Each day they had small amounts of orange juice,

grape juice, onions, and lettuce; and twice a week, very small portions of meat, fish or poultry.

The caloric intake was not restricted, the subjects being urged to try to keep their weight constant. The average intake was from 2500 to 3000 calories daily. Every attempt was made to keep the protein intake adequate, especially by consumption of cheese. In addition, the subjects took daily doses of halibut liver oil[1] containing 8500 I.U. of vitamin A and 1700 I.U. of vitamin D, and 50 mgm. of ascorbic acid.[1] By present standards and tables (4, 5) the diet was adequate in riboflavin, certainly deficient in thiamine, and presumably deficient in nicotinic acid, pantothenic acid and pyridoxine. However, data on these last three are unsatisfactory, nor are subsistence requirements for them well known. Therefore, the present paper deals mainly with the specific problem of thiamine deficiency.

At the end of the deficient period, each subject began taking thirty-six grams per day of Standard Brands' brewers' yeast type 2019,[1] for which the analysis is:

Thiamine	200–300 micrograms per gram
Riboflavin	70 micrograms per gram
Nicotinic acid	600 micrograms per gram
Pantothenic acid	200 micrograms per gram
Pyridoxine	85 micrograms per gram
Protein	45 grams per 100 grams

A. *Observations on the subject at rest.* *a.* Samples of urine were collected for 24 hours frequently. Their thiamine content was estimated by the method of Egaña and Meiklejohn (7). *b.* At the end of the normal period before and at the end of the deficient period, thiamine saturation tests were made after the method of Robinson, Melnick and Field (23). The subject took 5 mgm. of thiamine hydrochloride by mouth and then collected two successive twelve-hour specimens of urine. This type of saturation test is indicative of deficiency when only a small percentage of the dose of thiamine is excreted. *c.* The urinary nitrogen excretion of three subjects was measured throughout all four periods in order to estimate the protein intake. Routine estimations were made for all subjects of plasma protein, erythrocytes, leukocytes, hemoglobin, cell volume, and differential count. *d.* Basal observations. His basal metabolic rate was estimated by the open circuit gasometer method. Urine was collected for estimating his basal nitrogen, lactate and pyruvate excretion. Venous blood was taken for estimation of sugar, lactate and pyruvate. *e.* Weight was measured at frequent intervals. *f.* Complete urinalyses were made on all urine samples. *g.* Careful watch was kept for dermatological signs. *h.* Electrocardiograms were taken in each period. *i.* For three subjects, vibration sense was tested quantitatively with the pallesthesiometer. *j.* For two subjects, estimations were made of the free thiamine and diphosphothiamine in blood cells and plasma.

[1] We thank Abbott Laboratories for a gift of ascorbic acid and halibut liver oil, and Standard Brands, Inc. for the brewers' yeast used in this work.

B. *Observations on responses to exercise.* The routine followed in these observations was changed in detail but not in principle after the first four subjects had been studied. We merely made more different observations on the last group of three subjects. The response to exercise was measured on a motor-driven treadmill as previously described (22), walking first for 15 minutes at 3.5 m.p.h. and then running to exhaustion at 7 m.p.h. on an 8.6 per cent grade. No subject was able to run longer than four and a half minutes. The measurements made during the work were ventilation, oxygen consumption, carbon dioxide excretion, and pulse rate. As soon as he was exhausted, the subject sat on a stool and his oxygen debt was estimated for ten minutes in three periods of 1, 2 and 7 minutes. At 5 minutes after the run, capillary blood was drawn for estimation of sugar and lactate, and blood pressure was taken at intervals. After this period of 10 minutes, the subject lay on a bed and his oxygen debt was measured up to one hour's recovery. Pulse rates were taken manually at intervals and venous blood was drawn at 15, 30, 45 and 60 minutes for estimation of sugar, lactate, and pyruvate. At the end of this period a sample of urine was obtained covering the whole period of exercise and recovery. Lactate, pyruvate and nitrogen were all estimated in these samples, and a complete urinalysis was made.

The various analytical procedures were: Gasometric analyses by standard methods; lactate by the method of Edwards (6); pyruvate by the method of Lu (18) with the additional precaution of stabilizing the pyruvate by iodoacetate after Bueding and Wortis (3); blood sugar by the method of Folin and Malmros (9); urinary nitrogen by the method of Keys (17); blood diphosphothiamine by the method of Goodhart and Sinclair (10).

Results. Out of the very large number of observations, there were some positive findings and many negative ones. No single subject showed all of the changes described below, but a majority did. In general, a single subject would show either no change in a particular function, or else he would react like the rest of the group, never showing the reverse of what other subjects did.

A. *Dietary observations.* Table 1 lists the changes in one subject in weight, daily urinary nitrogen excretion, and daily thiamine excretion in the different periods. Conclusions to be drawn from these data for all subjects are: (1) Caloric intake was adequate in three cases; the maximal weight loss in any of the other subjects was 8 pounds. (2) Protein intake was adequate, at least 60–80 grams per day as calculated from the urinary nitrogen, the proteins consumed being mostly first-class proteins. (3) Thiamine deficiency was definite within four weeks, as measured by daily thiamine excretion (see fig. 1 and table 1) and by the thiamine saturation tests which are summarized in table 2. It is seen that the percentage of the test dose excreted in 24 hours was invariably much smaller in the deficient period than in the normal period before. (4) Administration of yeast was followed by restoration to very high levels of the excretion of thiamine daily.

B. *Observations on the subject at rest. a.* In agreement with the observations of other students of early B complex deficiency (15, 29), the symptoms of our

subjects were somewhat ill defined. A general feeling of lack of well being was noticed by five subjects, easy fatigue by five, loss of efficiency in the daily work

TABLE 1

Significant positive and negative findings in sedentary subjects

	SUBJECT	PERIOD						TYPE OF MEASUREMENT
		Normal before	Deficient			Yeast	Normal after	
			Day					
			7	14	21	3	10	
Weight (kilos)	R. D.	92.6	92.3	91.5	91.3	90.7	91.3	92.5	Nutritional state
Nitrogen excretion (grams in 24 hours)	R. D.	15.7	11.1	10.0	10.0	11.2	15.0	15.3	
Thiamine excretion (micrograms/24 hrs.)	R. D.	64	17	10	0	250	730	200	
BMR (calories/sq.m. and hr.)	J. W.	35.2	36.3	35.8	34.4	36.6	36.3	38.7	Basal data
Non-protein RQ	J. W.	0.85	0.81	0.79	0.76	0.80	0.84	0.85	
% of BMR due to carbohydrate	J. W.	37	31	25	16	26	39	40	
Blood lactate (mg. %)	J. W.	9	8	7	8	7	11	11	
Blood pyruvate (mg. %)	R. D.	0.6	1.0	1.0	1.0	1.6	1.2	1.0	
Ventilation (cc./kilo and min.)	R. D.	670	680	700	720	690	680	670	Walking uphill
Oxygen consumption (cc./kilo and min.)	R. D.	27.7	27.0	28.0	28.8	28.0	26.4	26.7	
Maximal heart rate	R. D.	167	161	164	165	173	165	163	
Maximal O_2 consumption	J. W.	41.7	45.3	43.8	44.4	45.2	43.6	45.0	Running uphill
Maximal CO_2 output	J. W.	55.5	57.4	54.0	54.6	55.2	55.0	55.2	
Maximal heart rate	J. W.	200	193	195	194	194	198	200	
Maximal blood sugar (mgm. %)	R. J.	156	137	126	128	124	132	134	Recovery after running
Maximal blood lactate (mgm. %)	J. W.	145	142	100	127	133	136	125	
Maximal blood pyruvate (mgm. %)	R. D.	4.9	5.0	4.6	4.9	5.1	4.7	4.9	
Oxygen debt in 1 hour (cc./kilo)	J. W.	169	161	174	157	149	157	162	

TABLE 2

Thiamine saturation tests

Each subject took 5 mgm. thiamine hydrochloride by mouth and then collected two successive samples of urine covering twelve hours each.

SUBJECT	THIAMINE EXCRETION IN 24 HOURS AFTER TEST DOSE	
	Normal before	Deficient
	micrograms	*micrograms*
Br	789	127
Da	795	109
Eg	746	241
He	365	118
Jo	460	97
Wh	708	116

by five, sleepiness, lethargy and lack of ambition by three, forgetfulness by two, constipation by one, poor appetite by two, irritability by two, paresthesias by two, gastro-intestinal upsets in the shape of slight nausea or diarrhea by two,

and muscle and joint pains during motion by one. The sum total of these symptoms is not striking; although none of the subjects felt perfectly well, none of them suffered acutely. After beginning to take yeast the subjects recovered relatively slowly and three subjects felt worse for a few days than at any time previously. By the end of the yeast period all felt perfectly well, even though they continued to eat the deficient diet. It was characteristic that certain subjects did not realize that they had been in poor condition until they improved after taking yeast. *b.* At the end of the deficient period, two subjects had notice-

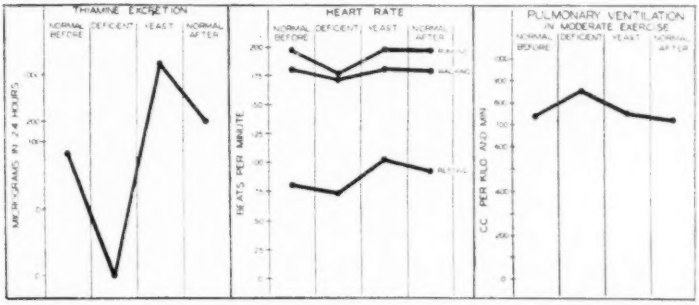

Fig. 1. Urinary excretion of thiamine, heart rate at rest and during activity, and pulmonary ventilation during moderate exertion, of sedentary subjects before, during and after deficiency in the vitamin B complex.

TABLE 3

Free thiamine and diphosphothiamine in the blood of subject unit

PERIODS	THIAMINE INTAKE	THIAMINE EXCRETION IN URINE	CELL VOLUME	DIPHOSPHO-THIAMINE IN CELLS	FREE THIAMINE IN CELLS	FREE THIAMINE IN PLASMA
	micrograms per day	*micrograms per day*	*per cent*	*micrograms per 100 ml. whole blood*	*micrograms per 100 ml. whole blood*	*micrograms per 100 ml. whole blood*
Normal before	1,000	85	39.2	7.2	2.4	1.8
Deficient	190	6	40.7	3.0	1.0	Trace
Yeast	10,171	1,354	41.0	3.5	2.7	0.7
Normal after	2,000	201	42.0	10.0	2.5	1.6

able cheilosis and scaling at the angles of the nose; these cleared up within a few days after taking the yeast. No subject had any signs within the mouth. *c.* No abnormality was observed in the three subjects on whom quantitative measurements of vibratory sense were made by the pallesthesiometer. *d.* The changes in thiamine and diphosphothiamine in the blood of subject W are shown in table 3. By the end of the deficient period when his urinary thiamine excretion was only 6 micrograms per day, the diphosphothiamine and the free thiamine in the cells and the free thiamine in the plasma had reached low levels. Even after 10 days of yeast, these values were not back to normal, except for the free

thiamine in the cells. Only after 10 days of normal diet were they all back to normal. This slow return corresponds to the slow disappearance of symptoms exhibited by this subject. *e*. The urine remained normal with respect to volume, specific gravity, albumin, sugar acetone bodies, and formed elements. *f*. There were no significant changes in the plasma proteins, erythrocytes, leukocytes, hemoglobin, cell volume or differential counts, with the exception of a tendency in two subjects to a slight normochromic normocytic anemia.

Electrocardiograms (leads 1, 2, 3, and 4-F) were taken on all subjects before, during and after the test period. Considerable care was taken to ensure uniformity of procedure. The tracings were always taken after a rest period with the subject recumbent and usually under strictly basal conditions. The electrocardiograph was tested for accuracy, the standardization recorded on each lead strip, and the same chest area was always used in taking lead 4-F. A minute analysis was made of the amplitude, form and duration of the several waves and intervals including the Q-T interval according to the formula:

$$Q - T = K\sqrt{\frac{\text{cycle}}{\text{length}}}$$

In 5 out of the 7 cases there were no significant differences in any of the electrocardiographic measurements; in the remaining 2 there were alterations in the T waves which require special comment. In one subject, the electrocardiogram taken before the test period shows a lower amplitude of the T waves which require special comment. In one subject, the electrocardiogram taken before the test period shows a lower amplitude of the T waves in leads 1 and 2 than at any time during the test period of one month or afterward. It was concluded that the vitamin deficiency which the subjects developed had no appreciable effect on the electrocardiogram. In one other subject there was slight but progressive lowering of the T waves in the limb leads during the test period. When yeast was added to the diet the T waves returned to their normal amplitude. Thus, in 7 subjects who were on a diet deficient in vitamin B, for periods up to one month, only one had electrocardiographic changes and these were of a minor character.

The experience of various investigators has not been uniform with respect to the electrocardiographic changes in patients with beriberi or in subjects with induced vitamin B-1 deficiency. Aalsmeer and Wenckebach (1) and Hashimoto (11), who have studied patients in the Orient with beriberi, have emphasized the fact that the electrocardiograms are rarely abnormal even in the presence of marked cardiac insufficiency. There were occasional exceptions to this rule and Hashimoto had described a case of acute pernicious beriberi in which negative T waves in lead 1 of the electrocradiogram became upright 50 hours after the administration of vitamin B-1. Minor changes in the electrocardiogram including sinus tachycardia, right axis deviation, and slight alterations in amplitude of QRS and T waves.

Significant changes in the electrocardiogram on the other hand have often been observed in patients with occidental beriberi (usually multiple vitamin de-

ficiency) (5). Kepler (16), in his oft-quoted case report, states that the electrocardiogram was normal, but inspection of the record reveals sagging of the RS-T segments in leads 1 and 2 and abnormally low amplitude of the T waves in all leads. Scott and Hermann (24) observed low voltage, inverted T waves and other abnormalities in the electrocardiograms from patients with beriberi and suggested that even in mild cases definite myocardial changes may occur. Weiss and Wilkins (28) observed abnormalities in the electrocardiograms from all but 5 of 57 patients with vitamin B deficiency but with no clinical evidence of heart disease. However many of the electrocardiographic alterations were of a minor character and most of their patients were in an age group when electrocardiographic abnormalities are frequently seen.

Observations on induced vitamin B-1 deficiency in man have shown (29) that although characteristic signs and symptoms develop they do not resemble beriberi in any of its typical forms. Only minor alterations in the electrocardiogram have been observed consisting chiefly in a diminution of the amplitude of the T waves. These alterations do not occur in all subjects and usually appear only after long periods of deficiency. They rapidly disappear after the administration of vitamin B-1. Individual variations, age of the subject, initial state of nutrition, the degree of activity, temperature of the environment, and nutritional deficiency other than vitamin B-1 are some of the variables which may account for the inconsistent results.

Our own experience leads us to conclude that in healthy young subjects, vitamin B (chiefly B-1) deficiency for short periods rarely causes significant changes in the electrocardiogram. The electrocardiographic alterations observed in patients with beriberi, especially in the Occident, may be due to prolonged nutritional deficiency, often in old patients with some degree of underlying heart disease. The rare occurrence of electrocardiographic abnormalities in patients with Oriental beriberi is difficult to explain, but may be due in part to the acute onset of the disease often in young and otherwise healthy subjects.

Basal functions (table 1 and fig. 1). *a.* The BMR decreased in the subjects who lost weight, and did not change in those who maintained their weight. The nonprotein RQ decreased steadily in two of the three subjects on whom it was measured most carefully, and increased again in the yeast period. The interpretation of this is obscure. It is the opposite of what is usually seen when a diet high in carbohydrate is eaten. *b.* The basal pulse rate decreased in four subjects who lost weight, and did not change in two who maintained weight. This is in agreement with the observations of Benedict et al. (2) on the effects of simple inanition. It is not the typical bradycardia of beriberi heart disease (28). The basal blood pressure was not changed. *c.* Basal blood sugar, blood lactate, blood pyruvate, and urinary lactate and pyruvate were not changed. These observations lend weight to the view that these measurements are of little diagnostic use in early thiamine deficiency, since changes in them are small and inconstant (25, 26, 29), in contrast to changes late in the course of thiamine deficiency in animals (8, 27), and in man (20).

C. *Observations on the response to exercise.* 1. Response to moderate work

(walking uphill). *a.* Functional cardiovascular alterations appeared in a majority of the subjects. In contrast to the tachycardia of beriberi and other types of thiamine deficiency, our subjects showed, during the deficient period, either their normal rise of the pulse rate during walking, or else an abnormally small rise. The pulse rate in recovery after walking likewise tended to be abnormally slow. These changes were reversed in the yeast period. The blood pressure showed no significant changes in any period or subject. *b.* The blood lactate and blood sugar showed no significant changes from the normal. *c.* The oxygen consumption and carbon dioxide excretion during this moderate work tended in most cases to increase slightly as deficiency progressed. The mechanical efficiency, measured by oxygen consumption, therefore was impaired by the deficiency (see table 1). This impairment was reversed when yeast was added to the deficient diet.

2. Response to exhausting work (running uphill). *a.* The duration of this run is an important measure of physical fitness. In the deficient period it was abnormally short in two of the three fittest subjects. More significant than this change is the fact that five of the six subjects were able to run longer in the yeast period than in either of the normal periods. *b.* The response of the blood pressure remained normal. The pulse rates showed significant alterations in the deficient period. In contrast to the tachycardia of beriberi (1, 28) our subjects tended to have an abnormally low maximal pulse, or else their usual response (table 1 and fig. 1). Even more significant was the fact that all subjects during the deficient period were abnormally slow in attaining their maximal heart rate during the run. These changes were all reversed in the yeast period. *c.* The maximal oxygen consumption and maximal carbon dioxide output remained the same in all periods (table 1). This suggests that no early defect in the oxidation-reduction systems of the muscles is to be found, and that the decarboxylating mechanisms remain unaffected in early deficiency. *d.* In contrast to animals (8) and man (12, 20) in late severe thiamine deficiency, our subjects had abnormally low maximal blood lactates, blood sugars, and urinary lactate and pyruvate excretions when deficient. Blood pyruvate was not affected.

3. Recovery after exhausting exercise. *a.* The blood pressure showed inconstant variations from subject to subject, and period to period. The pulse rates were either normal or slightly slower. *b.* There were neither significant changes in the total oxygen debt, measured for one hour, and its rate of repayment, nor in the total carbon dioxide excretion and its rate of excretion. *c.* The rates of removal of excess lactic acid, pyruvic acid and sugar from the blood stream, calculated according to the equation of Margaria and Edwards (19), were unaffected by the deficiency, although the maximal levels were lower in the deficient period. This finding contrasts markedly with what is seen in human beriberi (12, 21) and in animals (8).

4. Quantitative variations in physical fitness before, during, and after deficiency. *a.* In moderate work: As deficiency progressed, moderate exercise tended to cause an abnormally high pulmonary ventilation. These changes are shown in detail for one subject exposed at intervals to moderate work in the

shape of running on the level at 7.0 m.p.h. (fig. 1). This increased ventilation is perhaps associated with the easy fatigue noticed by the subjects, and may perhaps be regarded as incipient dyspnea on exertion. There tended to be a progressive decrease in mechanical efficiency in easy work as described above. This decrease was reversed by addition of yeast to the diet. *b.* In exhausting work: A quantitative index of fitness for hard exertion has been described by Johnson, Brouha and Darling (13). When applied to our subjects, it shows that in the deficient period there was impairment of fitness in four subjects and that all subjects improved in the yeast period (table 3). In fact, most of them were fitter in the yeast period than in the first or second normal periods. *c.* In repeated exhausting work: One of the most significant symptoms, noted especially by the two fittest subjects, was that after a single bout of exhausting exercise in the deficient period, subjective recovery before a second bout one

TABLE 4

Physical fitness for hard work

(Expressed as index of fitness according to Johnson, Brouha and Darling, poor being below 40; good, above 75)

SUBJECT	NORMAL BEFORE	DEFICIENT	YEAST	NORMAL AFTER
Jo*				
First run	63	60	68	74
Second run, 1 hour later		68	85	
Wh*				
First run	47	56	58	50
Second run, 1 hour later		45	58	
He	38	33	37	32
Bl	37	30	45	37
Da*	22	21	26	28
Eg	17	17	24	20

* These subjects exercised at least twice a week throughout the experiment.

hour later, and actual performance of the second bout, were abnormally poor. This phenomenon was measured and the fitness indices for the first and second exhausting runs in each period are shown in table 4. It is seen that the first run was better in the yeast period than in the deficient period; the second run was much better in the yeast period than in the deficient period; in comparison to the first run, the second was much better in the yeast than in the deficient period. It is worth mentioning that these measurable improvements agreed very well with the subjects' own feelings in the two periods. The practical conclusions may be drawn that B complex deficiency leads to diminution of fitness for a single bout of exhausting work; to an even more marked unfitness for repeated exhausting work, and therefore that the processes of recuperation after exercise are impaired by the deficiency.

Discussion. Early deficiency of the vitamin B complex in our subjects was accompanied within four weeks by regular changes only in the following three

ways: 1. The urinary excretion of thiamine dropped to low levels, and the percentage of thiamine excreted after a test dose was lowered. 2. Most of the subjects had mild symptoms. 3. There was moderate deterioration in physical fitness characterized by an abnormal increase in the pulmonary ventilation during moderate work, by a decreased ability to withstand exhausting work and especially by a decreased capacity for repeated exhausting work, reflecting clearly a lack of adequate recuperation.

All of the other measurements both cardiovascular and metabolic, showed no regular or constant alteration from period to period. In particular there were no characteristic changes in the electrocardiogram, in the blood pressure, blood lactate, blood pyruvate, blood sugar, urine lactate, or urine pyruvate at rest, during or after exercise. In other words, none of the metabolic and cardiovascular signs found in *late deficiency* can be relied upon to detect *early deficiency*. In general, it can be said that symptoms and signs relieved by yeast are very likely due to previous deficiency in the B complex.

The moderate deterioration in our sedentary subjects is strikingly different from what is seen in manual laborers exposed to a diet deficient in the B complex, who suffer within 5 days serious impairment of their fitness for sustained hard work and complain of acute symptoms (14).

SUMMARY

1. Seven healthy physicians subsisted on a diet deficient in the vitamin B complex, but adequate in calories and in proteins, for periods up to four weeks, then added brewers' yeast to this diet for two weeks, and finally reverted to a normal diet.

2. Deficiency, at least of thiamine, within 4 weeks was demonstrated by analysis of the daily thiamine output and by the rate of excretion of test doses of thiamine.

3. The symptoms were mild and vague, the most constant being easy fatigue, loss of ambition and loss of efficiency in daily work.

4. There was moderate deterioration of the subjects' physical fitness for exhausting exercise, and, particularly, poor recuperation between repeated bouts of exhausting exercise.

5. The above changes were the only regular findings in the deficiency, and they were all reversed by addition of brewers' yeast to the diet.

6. All other metabolic measurements showed slight abnormal changes or none at all in rest, moderate exercise, exhausting exercise and after exhausting exercise. These measurements were oxygen consumption, carbon dioxide excretion, blood lactate, blood pyruvate, blood sugar, urine lactate and urine pyruvate.

7. The cardiovascular changes were inconstant. There was never a tachycardia on exertion. On the contrary, the subjects tended to have abnormally slow heart rates in moderate exercise and in exhausting exercise. There were no abnormal changes in the blood pressure. In only one subject did the electrocardiograph show significant changes.

8. It is emphasized that under the conditions of these experiments only the

amounts of vitamins found in the urine, and symptoms and signs suggesting deterioration of the efficiency of the whole organism can be relied on to detect early deficiency.

9. Symptoms and signs that are cleared up by administration of brewers' yeast in adequate amounts are suggestive of deficiency in the B complex.

10. These findings on sedentary subjects are contrasted with rapid and striking effects of vitamin B complex deficiency in men doing daily hard work.

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THE ACID-BASE EQUILIBRIUM OF THE BLOOD IN EXERCISE

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Received for publication May 4, 1942

An increased blood lactate in humans during exercise is accompanied by a decrease in base bound as bicarbonate consequently causing a decrease in the CO_2 combining capacity of the blood. The reaction

$$BHCO_3 + \text{Lactic acid} \rightarrow B(\text{lactate}) + H_2CO_3$$

and the associated extra output of CO_2 through the lungs acts as one of the principal buffering mechanisms of the body. However observations upon the relation of the magnitude of changes in lactate and CO_2 capacity have been contradictory. Mellanby and Thomas (1920) and Evans (1922), by addition of lactic acid to drawn blood, found that the decline in CO_2 content was less than the increase in blood lactate. Results of similar experiments performed in this laboratory have shown close agreement yet the picture is not identical with that seen in blood drawn after exercise. In six observations on blood drawn after exercise, Barr, Himwich and Green (1923) obtained wide variations and found a greater change in blood lactate than in CO_2 capacity in only two cases. Dill, Talbott and Edwards (1930) found in general a greater decline in CO_2 capacity of the blood. Dennig et al. (1931) found approximately equal changes when the blood lactate rose to 10 mEq. per liter. Robinson and Harmon (1941) found that the decreases in CO_2 capacity, at physiologically high concentrations of blood lactate, were smaller than the corresponding increases in lactate.

By further study of this problem we have attempted to relate changes in blood lactate, CO_2 capacity, and serum pH and to determine the rôle of the various mechanisms in buffering acid as it is accumulated during exercise. Samples of blood were drawn from human subjects in the basal state and after exercise on the same day. Various intensities of exercise, which consisted of running on a motor driven treadmill or in competitive races, were used to produce different concentrations of blood lactic acid in the men. For comparison of changes in pH, CO_2 capacity, lactate, and related changes in available base, arterial blood samples were drawn under oil and treated with heparin. Several samples of venous blood were used in the comparison of the variations in lactate concentration with those of CO_2 capacity. Blood lactate was determined by the method of Edwards (1938), and plasma protein by micro-Kjeldahl analysis. HbO_2 and CO_2 capacity were determined by equilibration of blood with O_2 and CO_2 pressures of 200 and 40 mm. Hg respectively at 37°C. as described by Dill in Henderson's book (1928). Analyses of blood samples for both content and capacity of HbO_2 and CO_2 were done on the Van Slyke apparatus. The pH values of arterial blood samples were calculated by means of the Henderson-Hasselbalch equation and some over the entire range of values

obtained were checked by the electrometric method as described by Dill, Daly and Forbes (1937). The two methods checked each other very closely. The term "CO_2 capacity" as used here is defined as the CO_2 content of oxygenated whole blood at 37°C. and 40 mm. Hg CO_2 tension.

RESULTS. Figure 1 reveals a distinct relationship between the increase in lactate (Δ lactate) and the decrease in CO_2 capacity (Δ CO_2 capacity). Increases of lactate up to 4 mEq. per liter are accompanied by approximately equivalent decreases in CO_2 capacity. In this range almost all of the base used in neutralization of the acid is obtained from base bound as bicarbonate. However, as the concentration of base bound as bicarbonate is decreased beyond this

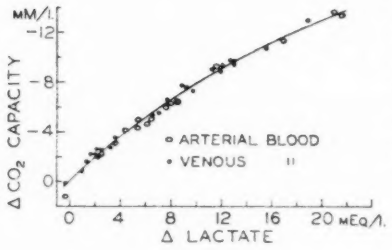

Fig. 1

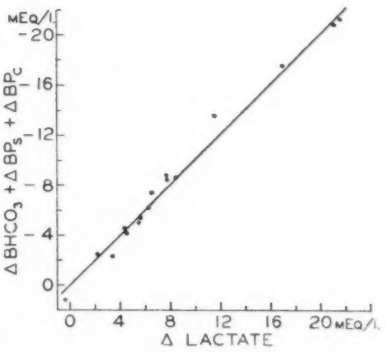

Fig. 2

Fig. 1. Increases above the basal level of blood lactate (Δ lactate) in relation to corresponding decreases in CO_2 capacity (Δ CO_2 capacity) of the blood of men after various intensities of exercise.

Fig. 2. Increases above the basal level of blood lactate (Δ lactate) plotted against corresponding decreases of available base from bicarbonate (Δ $BHCO_3$), serum proteins (Δ BP_s) and cell proteins (Δ BP_c) in arterial blood drawn after various intensities of exercise. The straight line indicates equal changes of lactate and base.

point by higher lactate concentrations the ratio of $\frac{\Delta\ CO_2\ capacity}{\Delta\ lactate}$ becomes progressively smaller. With the accumulation of lactic acid the pH of the blood decreases and approaches the isoelectric point of the blood proteins decreasing their base binding capacity and releasing base for neutralizing the acid. Thus as the concentration of lactic acid becomes higher the plasma proteins and hemoglobin account for a greater fraction of the base used to neutralize the accumulated acid.

Calculations of changes in the arterial blood samples have revealed the relation of increases in lactate concentration to decreases in available base from bicarbonate, cell proteins, and plasma proteins. Decreases in base bound as bicarbonate in the whole blood $(\Delta\ BHCO_3)_b$ were calculated from values of the

CO_2 tension and content. Base contributed by plasma proteins (Δ BP_s) has been calculated from the data of Van Slyke, Hastings, Hiller and Sendroy (1928).

$$BP_s = 0.104 \text{ (gram protein) } (pH_s - 5.08)$$

Grams of plasma proteins per liter of blood were calculated from protein analysis of plasma and hematocrit determination of plasma and cell volumes. Similarly the decrease in base bound by protein in the cells (Δ BP_c) has been calcu-

TABLE 1

Arterial blood changes in exercise

	ΔLACTATE mEq./l.	ΔCO_2 CAPACITY mM./l.	ΔpH_s	$\Delta(BHCO_3)_b$ mEq./l.	ΔBP_c mEq./l.	ΔBP_s mEq./l.
1	−0.3	1.2	0.03	1.0	0.14	0.12
2	2.2	−2.1	−0.03	−2.3	−0.13	−0.04
3	3.5	−3.1	−0.07	−1.8	−0.25	−0.21
4	4.5	−4.1	−0.05	−3.5	−0.48	−0.17
5	5.5	−4.3	−0.09	−3.8	−0.83	−0.32
6	5.5	−5.0	−0.11	−4.3	−0.68	−0.37
7	6.2	−4.6	−0.11	−4.8	−0.91	−0.37
8	6.5	−5.1	−0.07	−6.5	−0.72	−0.24
9	7.7	−6.1	−0.11	−7.1	−1.30	−0.48
10	7.8	−6.4	−0.07	−6.7	−1.25	−0.46
11	8.5	−6.5	−0.14	−6.7	−1.50	−0.41
12	11.6	−9.0	−0.18	−11.0	−1.86	−0.71
13	17.0	−11.5	−0.30	−13.1	−3.23	−1.12
14	21.1	−13.6	−0.38	−15.3	−4.05	−1.49
15	21.6	−13.5	−0.40	−15.5	−4.14	−1.70

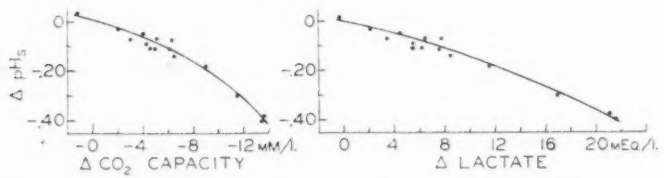

Fig. 3. The relationship of increases above the basal level of blood lactate (Δ lactate) and decreases in CO_2 capacity (Δ CO_2 capacity) to the decline of pH_s (Δ pH_s) of arterial blood after various intensities of exercise.

lated from the formula for oxygenated cells derived by Dill, Edwards and Consolazio (1937).

$$BP_c = HbO_2[-0.5\ (pH_c)^2 + 10.625pH_c - 48.46]$$

In table 1 have been tabulated the changes found in 15 arterial blood samples drawn after exercise. The increases in blood lactic acid (Δ lactate) are about equivalent to corresponding combined decreases in available base according to the equation

$$\Delta \text{ lactate} = \Delta(BHCO_3)_b + \Delta BP_s + \Delta BP_c$$

This relationship is represented in figure 2.

Figure 3 shows changes in the pH of arterial blood serum (ΔpH_s) as related to Δ lactate and Δ CO_2 capacity. Throughout the range of lactate concentrations studied the ratio of $\frac{\Delta\ pH_s}{\Delta\ \text{lactate}}$ is almost constant being only slightly greater at high values of lactate. In contrast the ratio of $\frac{\Delta\ pH_s}{\Delta\ CO_2\ \text{capacity}}$ steadily increases as the CO_2 capacity is lowered. This again demonstrates the decreased buffering action of bicarbonate as the concentration is lowered.

The maximum ΔpH_s of -0.40 pH units was a decrease, measured in a well-trained athlete, from a basal pH_s value of 7.37 to a pH_s of 6.97 after work. Bock, Field and Adair (1923) and others have measured similarly low pH_s values in diabetic coma. It has been our experience that lactate values of 22 mEq. per liter are not uncommon in trained athletes after hard races. In such cases the arterial pH_s probably drops to about 7.0 yet these athletes show no ill effects aside from a breathlessness after the race from which they soon recover.

SUMMARY

Blood samples drawn from human subjects in the basal state and after various intensities of exercise were analyzed for O_2 and CO_2 capacity, lactic acid, plasma proteins, and serum pH. Comparison of increases in lactate concentration (Δ lactate) and decreases in CO_2 capacity (Δ CO_2 capacity) up to 4 mEq. per liter showed approximately equivalent changes. Beyond this point decreases in CO_2 capacity became progressively smaller than corresponding increases in lactate concentration. Base for neutralization of the lactic acid at low concentrations was obtained principally from bicarbonate. At the higher lactate concentrations hemoglobin and plasma proteins accounted for an increased fraction of this base. Decreases in serum pH of arterial blood (Δ pH_s) varied directly with Δ lactate and Δ CO_2 capacity. The maximum blood lactate value measured was 22 mEq. per liter with a corresponding serum pH of 6.97.

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SEXUAL BEHAVIOR IN RATS WITH LESIONS IN THE ANTERIOR HYPOTHALAMUS

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Received for publication July 6, 1942

In 1938 Fisher, Magoun and Ranson (1) reported that cats with small electrolytic lesions in the anterior hypothalamus failed to come into heat and that if such lesions were placed in pregnant cats abnormal deliveries or failure to deliver occurred. These results led the writer to test the sexual behavior of a group of rats which had been prepared for a study in diabetes insipidus (2). The findings in these rats were later amplified by the preparation of another series. In all of these animals electrolytic lesions were placed in the anterior hypothalamus with the aid of the Horsley-Clarke stereotaxic instrument (3) as modified for the rat (4). No changes in sexual behavior were noted in those rats with bilateral lesions in the lateral half of the anterior hypothalamus. In some of the rats with medially placed lesions, however, there was found constant vaginal estrus with refusal to mate while a few others exhibited constant vaginal diestrus. Only a few of the rats studied were males but, although the lesions were quite minute, an occasional rat refused to mate. The location of the lesions and the behavior of some of these rats were apparently quite similar to those of the male and female guinea pigs reported by Dey et al. (5, 6, 7, 8, 9, 10).

In all of the rats with electrolytic lesions which showed sexual abnormalities the damage included portions of the medial half of the anterior hypothalamus but many with lesions in this region seemed quite normal and it proved to be impossible to correlate symptoms with lesions. Since the possibility existed that the sexual abnormalities found could be explained on the basis of pituitary damage even though this gland were far removed from the anatomical limits of the lesion (Ingram, (11) has suggested a similar explanation for disturbances in carbohydrate metabolism following hypothalamic lesions in cats) it was decided to attempt the destruction of portions of the anteromedial hypothalamus by some other method which might minimize this possible damage.

The following procedure was finally adopted. Using a small dental crown saw, a small cut was made in the calvarium just caudad of the coronal suture. Through this opening a knife was plunged at a predetermined angle to the base of the brain. The first knife used was prepared from a thin dental spatula 3 mm. wide with a rectangular end. Later a 4 mm. instrument was made from a thin razor blade and a wire guide was used to insure the proper angle. In most cases the plane of section passed caudad of the anterior commissure and reached the base of the brain between the middle of the optic chiasma and the anterior border of the median eminence. The high mortality (over 50 per cent) perhaps precludes the use of this method in other animals.

The first five males and all the females were allowed to recuperate from the

immediate effects of the operation and were then placed with animals of the opposite sex in large cages. Daily vaginal smears were made on the females until a pregnancy sign was found or until the animals were sacrificed while the males were observed at irregular intervals. After recovery from the immediate effects of the operation the remaining thirteen males, which were not sexually mature at the time of operation, were placed with females of the same age in small cages (1 male and 1 female per cage). Those males which sired normal litters were sacrificed while the remainder (10) were rotated between large cages with four females and small cages with one female. All these normal females were smeared daily. Two males were sacrificed when plugs or sperm were found in the vaginal smears of the females they were with. This left eight rats which had been with from 10 to 13 different normal females during 20 to 30 periods of estrus. For a terminal experiment eight normal females whose AM smear consisted only of round nucleated cells were chosen. Beginning at 11:30 a.m. these were tested for heat at hourly intervals by placing a normal male with them. After the first female came into heat (3:30 p.m.) the remaining eight experimental males were rotated repeatedly through the cages containing the females in heat. There were two cages with three females and one cage with two females. Each male was left with a group of females for ten minutes. At 6:30 p.m. all the females were in heat (i.e., had accepted normal males). There was only one male, a markedly obese animal, which did not make the preliminary investigations such as are made by normal males—smelling and licking the external genitalia of the females, ruffing the hair of the shoulder region, etc. This was done, however, in a rather cursory manner and they did not show the intense excitement such as is usually seen in normal males. In an attempt to increase the sexual excitement of the females and thus, possibly, that of the experimental males, normal males were placed with the females and allowed to copulate once or twice. This procedure markedly increased the excitement of the females but affected only one male. The exception, a slightly stunted individual, which previously had shown only perfunctory interest in the females began mounting and copulating. For the remainder of the test period this rat's activity was almost identical with that of a normal male. By 9 p.m. when the experiment was concluded each of the males had had at least 30 chances (times with females times the number of females per cage) to mate.

There were only five females in the series. All of these ran normal sexual cycles and four became pregnant and delivered litters. Only one of the four suckled her young, however. Since the behavior of the other three seemed comparable to that of the occasional young female which refuses to care for her first litter these three were bred several times but they repeatedly refused to suckle their young. These three were sacrificed three days after the delivery of their final litters. Their mammary glands were fixed after the manner of Jeffers (12) and their glands were compared with those of a normal female which was sacrificed three days after delivery and whose litter was killed when born. Histologically there was no difference between the glands of the normal and the experimental animals. Sperm and plugs were repeatedly found in the vaginal

smears of the one rat which failed to become pregnant, but neither pregnancy nor pseudopregnancy resulted. Despite repeated attempts (sometimes two males of proved potency were kept in the cage with this female) no changes in the length of the cycle occurred.

All the animals were killed by decapitation, the brains were removed and fixed in formalin, the reproductive organs were fixed in Bouin's and all the tissues were embedded in paraffin. A few of the brains were sectioned transversely, in these it was difficult to determine if the lesion reached the base of the brain; others were sectioned sagittally, in these the lateral limits were hard to define; the remainder and most satisfactory were sectioned horizontally. All were mounted serially and stained with cresyl violet.

A study of the brains of these rats, in contrast to that of the animals with electrolytic lesions, was quite instructive. If one considers first those animals which showed definite abnormalities of sexual behavior one must be guided by the

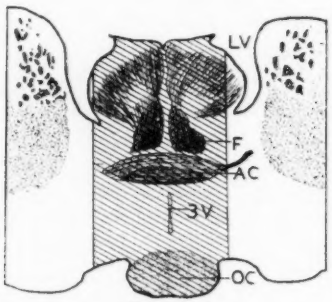

Fig. 1

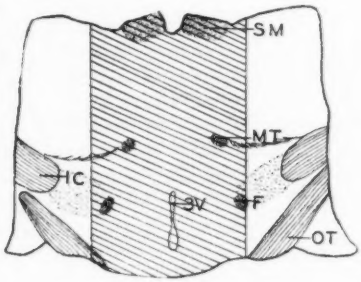

Fig. 2

Figs. 1 and 2. Diagrammatic reconstructions of the most rostral (fig. 1) and the most caudal (fig. 2) lesions in the group of rats with symmetrical lesions and normal sexual behavior. Abbreviations as follows: *AC*, anterior commissure; *IC*, internal capsule; *F*, fornix; *LV*, lateral ventricle; *MT*, mammilothalamic tract; *OC*, optic chiasma; *OT*, optic tract; *SM*, stria medullaris; *3V*, third ventricle.

principle that the smallest lesion which abolishes a given set of responses is the most important. Unilateral damage failed to abolish sexual behavior but several males whose lesions barely crossed the midline failed to copulate. This may perhaps indicate that some structure concerned in the integration of sexual behavior may lie adjacent to the third ventricle. However we occasionally find in our colony a male or female rat, otherwise normal, which refuses to copulate or to become pregnant. If one approaches the problem from the other angle, that is, if one studies only those rats which showed normal or approximately normal sexual behavior then one may discard all animals with asymmetrical lesions and one does not have the normal variation in sexual drive to consider. There were ten rats in this group, 8 males and 2 females. The lesions of two of these are shown diagrammatically in figures 1 and 2. In making these drawings the horizontal sections were projected upon appropriate transverse sections from the brain of a normal rat.

These two lesions are the most rostral (fig. 1) and the most caudal (fig. 2) of the group. Laterally they extend beyond the fornices but their exact ventral extent was difficult to determine and it is possible that at least some of the fibers occupying a very superficial position on the base of the brain may have escaped destruction. The lesions in the remaining eight animals were quite similar in extent and lay at intervals between the two shown in the figures. It should be emphasized that the behavior of these rats could not be predicted from the lesions. Two rats, one of which failed to mate and another which seemed to be quite normal, might have almost identical lesions.

Several conclusions may be drawn from these findings. First, there seemed to be a tendency for damage to the medial half of the anterior hypothalamus to depress sexual activity. Second, transverse lesions extending from fornix to fornix and lying at various levels from the middle of the optic chiasma to the anterior border of the median eminence are not incompatible with normal sexual behavior. Third, if there is in this region any area or areas essential for normal sexual behavior, fibers to and from this "center" must pass by one of two routes: 1, directly ventrad and pursue a very superficial course therefrom; and 2, directly laterad to beyond the fornices. Fourth, if there is any such structure its connections must be rather diffuse as it is probable that in at least one of these rats this "center" must have been bisected.

This work, then, neither affirms nor denies the possibility that there may exist in the medial half of the anterior hypothalamus a structure or structures essential for the integration of normal sexual behavior. It does indicate the improbability that such is the case and very definitely limits the course of fibers to and from this hypothetical center.

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RADIOACTIVE PHOSPHORUS STUDIES ON STRIATED AND CARDIAC MUSCLE METABOLISM[1]

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From the Department of Pharmacology, University of Michigan Medical School, Ann Arbor

Received for publication July 10, 1942

It has been demonstrated by the use of radioactive phosphorus (P^{32}) that the formation of lactic acid in contraction of striated muscle does not involve the interchanges of phosphate groups postulated by the currently accepted mechanism for glycolysis (8). For the isotope technique to yield results of critical value, it is essential that the resting metabolism of the muscles lead to a differential distribution of the P^{32} among the organic compounds present. Fortunately, the conditions chosen did result in such a differential distribution.

The present work was undertaken primarily to study the time course of the distribution of P^{32} in the resting metabolism of muscle, and thereby to determine the basis for the differential effects previously found. The data so obtained might be expected to indicate whether the postulated phosphorylating glycolysis may be operating in the resting metabolism of the intact cell, even though it does not function in the activity metabolism. Observations were also made on the distribution of P^{32} in the intact beating heart, for comparison with the data of Barker, Furchgott and Shorr (2) on quiescent heart slices.

Experiments were carried out on frogs and cats. The general procedure was to inject the P^{32}, in the form of Na_2HPO_4, into the ventral lymph sac in frogs, and subcutaneously in cats, then, after the lapse of one or more hours, to freeze the tissues in situ. The various P compounds were separated from trichloroacetic acid filtrates of the powdered tissues, the P converted to $MgNH_4PO_4$, and measurements of the P^{32} content per millimeter P were then made. Data were obtained on striated muscle of frogs at 1, 2, 24 and 48 hours after injection of the P^{32}, and on striated muscle, cardiac muscle, and plasma of cats at 1, 2, 4 and 24 hours after injection. Determinations of P^{32} content were made on inorganic P, phosphocreatine (PC), the two readily hydrolyzable groups of adenosine triphosphate (ATP), the difficultly hydrolyzable one of the adenylic acid residue (AA), the hexosemonophosphate (HMP) of striated muscle and heart, and on the plasma inorganic P.

The frogs were anesthetized with urethane for the short periods of observation; for the longer ones, they were decerebrated a day in advance. Immediately after the injection of the P^{32} they were placed in small wire cages in running water; at the proper time cage and contents were dropped into the freezing mixture. The muscles of both hind legs were taken together.

The cats were anesthetized with pentobarbital just prior to the injection of the P^{32}. In the 24-hour group, the anesthesia was administered 2 hours before the tissues were to be sampled. Since the previous work (8) had shown that

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a tetanic contraction does not alter the P^{32} content of the PC or ATP, it was felt that the small amount of muscular activity shown by cats confined to cages would not vitiate the results. The gastrocnemius muscles were prepared for freezing in the usual way, the trachea was cannulated, and one carotid artery exposed for cannulation. At the proper time the muscles were frozen, the artery cannulated, and blood run directly into a flask containing crystalline heparin. The thorax and pericardial sac were then opened under artificial ventilation, and the freezing mixture poured over the heart. The time elapsed from freezing the first muscle to freezing the heart was between 4 and 6 minutes.

The phosphorus compounds were separated from the trichloroacetic acid filtrates by the methods previously described (8). The procedure for the isolation of the P present in the different forms was modified somewhat. One portion of the filtrate was treated with magnesia mixture made up with magnesium and ammonium nitrates, precipitating the inorganic phosphate; the PC in the filtrate from this was hydrolyzed by adding nitric acid to 2 N, excess ammonium molybdate, and letting stand 1½ hours at room temperature. Another portion of the trichloroacetic acid filtrate was treated with barium hydroxide for the separation and isolation of ATP and HMP. The Ba precipitate, containing inorganic phosphate, ATP and adsorbed PC, was dissolved in 2N nitric acid, ammonium nitrate added to a concentration of 5 per cent, and excess ammonium molybdate added. This was let stand 1½ hours at room temperature to precipitate inorganic phosphate and that liberated by the hydrolysis of the adsorbed PC; this precipitate was discarded, and the filtrate heated 20 minutes in a boiling water bath to separate the two readily hydrolyzable groups of the ATP. The phosphate group of the AA was separated by heating the filtrate from the previous precipitation on the steam bath for 24 hours. The HMP was separated from the barium hydroxide filtrate. This was made approximately 1 N with sulfuric acid, the precipitated $BaSO_4$ removed by centrifugation, and the supernatant let stand over night to hydrolyze the PC. This phosphate was precipitated by magnesia mixture, and the HMP in the filtrate broken down by wet ashing with sulfuric and nitric acids. The inorganic phosphate formed was then precipitated with magnesia mixture. Plasma inorganic phosphate was precipitated by magnesia mixture from trichloroacetic acid filtrates.

All the phosphate precipitates were converted to $MgNH_4PO_4$, washed well, and dissolved in dilute nitric acid. Small aliquots were taken for determination of P, and the major aliquots transferred to 5 ml. beakers (those used with the Beckman pH meter) and evaporated to dryness. Determinations of relative P^{32} content were made with a Geiger-Müller counter, using a glass bubble type of Geiger-Müller tube with a background count of 10 per minute. Control observations showed that the errors due to non-uniform distribution of the evaporation residue on the bottom of the beaker were negligible. The activity of the experimental samples ranged from 2 or 3 net counts per minute up to several hundred. The counting period was at least 8 minutes for even the most active samples; for those of low activity the counting period was sufficiently prolonged to give a minimum of 200 net counts above background. The meas-

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urements on these samples are probably accurate to within 10 per cent; on the the active samples the accuracy of the counting is between 2 and 3 per cent.

The quantities of P^{32} injected, per kilogram body weight of the animals, were such as corresponded to between 5×10^5 and 2×10^6 counts per minute with the Geiger-Müller tube used. The total amounts of phosphorus injected were naturally subject to considerable variation. In the tables of data, all measurements have been corrected for decay to a standard time for each animal, and have been recalculated to the basis of 1×10^6 counts per minute per kilogram body weight. Thus the data on any two animals are directly comparable.

The data on the cat muscles are given in table 1. Comparison of the P^{32} contents of the PC and ATP with that of plasma inorganic phosphate shows that the rate of exchange of these compounds with plasma phosphate is quite low. At 2 hours after the injection the apparent turnover is only 1 part in 200. The turnover rate of the ATP is somewhat lower than that of the PC. The turnover rate of the AA is about one-fourth that of the two readily hydrolyzable groups of the ATP.

The HMP fraction shows a peculiar behavior in that 2 hours after the injection, the P^{32} content of this fraction is three times as high as in the PC or ATP, but at 4 hours it has dropped to a small fraction of this value while the PC and ATP have increased their P^{32} contents slightly. At 24 hours the relation between the HMP and the PC and ATP is essentially the same as at 4 hours. These findings can be explained on the basis that, in addition to the expected metabolic turnover of HMP, a temporary accumulation of this substance takes place on the cell membrane when a large amount of phosphate is injected. The material on the membrane is hydrolyzed, with liberation of the phosphate into the extracellular phase, as the phosphate is excreted. The metabolic turnover should be independent of the absolute amount of phosphate injected, whereas the accumulation on the membrane might be expected to depend on this absolute amount.

To test this hypothesis, the 2-hour experiments were repeated with a sample of P^{32} which had been subjected to prolonged intensive bombardment, so that an adequate amount of radioactivity was contained in a fraction of a milligram of P, instead of in the much larger amounts that had previously been used. The results, which are given in table 2, are those which would have been anticipated from the above hypothesis. The P^{32} contents of plasma inorganic phosphate, the PC and the ATP are the same as when the large amount of phosphate was given, but the P^{32} content of the HMP is well below that of the other two organic compounds, so that it is comparable to the 4-hour experiments. These data show that the turnover rates of the PC and ATP are independent of the absolute amount of phosphate injected, and also show the HMP does not exchange with other intracellular components.

Turning now to the organic compounds of the heart muscle, in table 3, it is obvious that the turnover rate with plasma phosphate is very much greater than in resting striated muscle. At 2 hours, for example, the P^{32} contents of the PC and ATP are over 20 times as great as in the striated muscle. The HMP

shows a lower P^{32} content than the other two compounds. The tremendous difference between heart and striated muscle is not due to the constant activity

TABLE 1

Distribution of radioactive phosphorus (P^{32}) in resting muscles of cats

Values are in terms of net counts per minute per milligram P, calculated to the basis of 1×10^6 counts per minute injected, per kilogram body weight.

PLASMA INORG. P	MUSCLE INORG. P	CALC. INTRACELLULAR INORG. P	PHOSPHOCREATINE	ADENOSINETRIPHOSPHATE	HEXOSE MONOPHOSPHATE	ADENYLIC ACID	PLASMA INORG. P	MUSCLE INORG. P	TOTAL P INJECTED
A. 1 hour after injection									
							mgm. per cent	*mgm. per cent*	*mgm. per kgm.*
17,100	303	*	34	48	40		6.5	25	9.7
14,050	395	36	34	49	17		5.6	24	9.8
15,500	415	*	21	22			6.3	16	9.0
7,800	260	*	16	10	26		7.9	15	9.1
Av.....13,612	343		26	32	28				
B. 2 hours after injection									
15,300	905	172	93	99	216		6.9	16	2.8
18,800	540	*	48	56	308		7.6	25	2.8
15,400	610	21	89	57	390	18	5.2	15	6.3
15,100	690	190	115	100	146	13	5.5	18	6.3
Av.....16,150	686		86	78	265	16			
C. 4 hours after injection									
5,300	420	218	95	82	43	30	7.2	20	8.5
7,150	224	37	63	50	28	18	6.0	25	8.5
15,000	485	29	142	94	30	17	5.0	18	2.8
11,250	442	110	175	113	27	26	4.9	21	2.9
Av..... 9,675	393	99	119	85	32	23			
D. 24 hours after injection									
547	169	159	126	117	57	39	5.2	22	11.2
915	154	132	114	101	37	18	6.0	22	11.2
1,055	153	120	125	121	51	15	5.6	17	8.5
1,115	126	93	99	67	60	11	5.6	19	8.5
690	188	169	145	157	58	52	5.6	17	9.1
735	248	230	191	181	120	50	5.6	16	9.1
Av..... 843	173	151	133	124	64	31			

* P^{32} content of extracellular fluid P less than that of plasma P.

of the former, for the data of Barker, Furchgott and Shorr (2) on quiescent heart slices show a very close correspondence to the present findings. They found that

at equilibrium the PC and ATP had about one-sixth the P^{32} content of the inorganic phosphate of the medium, and that of the HMP was considerably lower. The 2-hour data here show practically this 1 to 6 ratio between the PC and ATP and plasma inorganic phosphate. The logical interpretation of this similarity of findings on the beating heart and the non-beating slices is that the contraction process in this organ, as in striated muscle, does not involve the phosphate interchanges of the glycolytic cycle.

Comparison of the data on frog muscles, in table 4, with those of the cat muscles shows that the apparent turnover rate and time course of the phosphate distribution are essentially the same in the two species. The data on the frogs 48 hours after injection emphasize that the HMP does not interchange with PC,

TABLE 2

Influence of absolute amount of P *injected on distribution of* P^{32} *in resting muscles of cats*

Values are in terms of net counts per minute per milligram P, calculated to the basis of 1×10^6 counts per minute injected, per kilogram body weight.

A. 2 hours after injection of large amount of P

P INJECTED	INORGANIC P	PHOSPHOCREATINE	ADENOSINE TRIPHOSPHATE	HEXOSEMONO-PHOSPHATE	PLASMA INORG. P
mgm. per kgm.					
2.8	905	93	99	216	15,300
2.8	540	48	56	308	18,800
6.3	610	89	57	390	15,400
6.3	690	115	100	146	15,100
Average.....	686	86	78	265	16,150

B. 2 hours after injection of small amount of P

0.25	620	45	58	17	19,900
0.25	790	86	74	29	11,700
0.31	870	97	78	39	13,500
0.31	680	71	95	38	12,500
Average.....	740	75	76	31	14,400

ATP or intracellular inorganic phosphate, since the ratio of the P^{32} contents is the same at 48 hours as at 24 hours.

With respect to the inorganic phosphate, the principal consideration is the mechanism by which phosphate enters the cell from the extracellular phase. It is generally recognized that the cell membrane of muscle is impermeable to anions, yet in studies of diffusion of phosphate by the isotope technique, such as those of Hevesy and Rebbe (4) and Manery and Bale (6), the assumption is made, explicitly or implicitly, that phosphate enters the cell by simple diffusion, and is then converted to the organic compounds. The present data make such a position untenable, and show that phosphate enters the cell of striated or cardiac muscle only by being converted to an organic compound, presumably at the membrane. Conversely, phosphate can leave the cell only by the hydrolysis of these organic compounds at the membrane. This will be made clear by a

comparison of the P^{32} contents of plasma and *intracellular* inorganic phosphate with those of the PC and ATP.

TABLE 3

Distribution of radioactive phosphorus (P^{32}) *in cardiac muscle of cats*

Values are in terms of net counts per minute per milligram of P, calculated to the basis of 1×10^6 counts per minute injected, per kilogram body weight.

PLASMA INORG. P	HEART INORG. P	CALC. INTRACELLULAR INORG. P	PHOSPHOCREATINE	ADENOSINETRIPHOSPHATE	HEXOSE MONOPHOSPHATE	ADENYLIC ACID	PLASMA INORG. P	HEART INORG. P
A. 1 hour after injection								
							mgm. per cent	*mgm. per cent*
17,100	3260	*	1730	1780	725	154	6.5	10
14,050	1225	*	645	720	315	64	5.6	13
15,500	1690	*	570	460	200	29	6.3	10
7,800	900	*	286	228			7.9	10
Av.....13,612	1769		808	797	413	82		
B. 2 hours after injection								
15,300	3100	392	1360	1200	390	640	6.9	12.5
18,800	3040	*	1985	2000	460	415	7.6	12
15,400	3010	82	2640	1870	1020	101	5.2	9
15,100	5680	1570	3160	3230	1090	110	5.5	6
Av.....16,150	3708		2286	2075	740	317		
C. 4 hours after injection								
5,300	1920	865	1515	1465	1105	88	7.2	10
7,150	2000	730	1550	1310	1210	103	6.0	10
15,000	4270	2845	3090	2860	690	650	5.0	14
11,250	3210	2530	2430	2390	760	455	4.9	21
Av..... 9,675	2850	1743	2146	2006	941	324		
D. 24 hours after injection								
547	1045	1150	980	990	650	600	5.2	10
915	1210	2100		1755	830	810	6.0	7
1055	1910	1930	1800	1670	2530	510	5.6	14
1115	1740	1840	1750	1880	1140	324	5.6	12
690	1340	1535	1145	1190	1050	475	5.6	8
735	1260	1390	1250	1380	1015	367	5.6	9
Av..... 843	1417	1654	1385	1478	1203	534		

* P^{32} content of extracellular fluid P less than that of plasma P.

The diffusion theory requires that the P^{32} content of the intracellular inorganic phosphate be higher than that of the PC or ATP while the plasma P^{32} is higher than that of the PC or ATP. It is obviously not possible to separate the intracellular inorganic phosphate from that in the extracellular phase, but the amount

present and its content of P^{32} can be calculated from the P contents of tissue and plasma, the P^{32} contents of plasma and tissue inorganic phosphate, and the volume of the extracellular phase. This last item has been determined by Amberson et al. (1) and by Yannet and Darrow (10), from the chloride contents of tissue and plasma. The two sets of data are in excellent agreement in assigning

TABLE 4

Distribution of radioactive phosphorus (P^{32}) *in resting muscles of frogs*

Values are in terms of net counts per minute per milligram P, calculated to the basis of 1×10^3 counts injected per gram body weight.

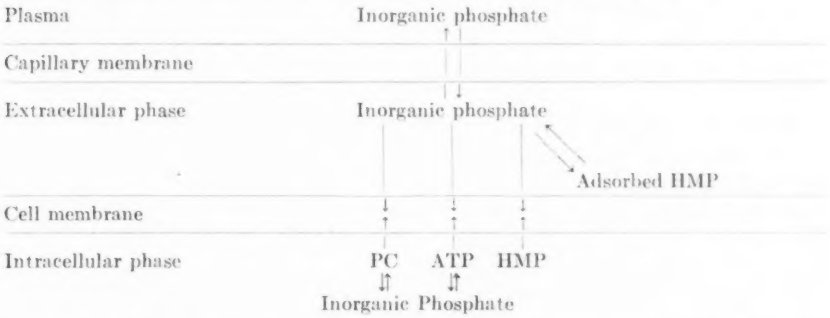
INORGANIC PHOSPHATE	PHOSPHOCREATINE	ADENOSINE TRIPHOSPHATE	HEXOSEMONOPHOSPHATE	ADENYLIC ACID	TOTAL P INJECTED GAMMA PER GRAM
		A. 1 hour after injection			
254	72	72	123		21
437	65	91	150		21
218	54	49	98		21
Av. 303	64	71	124		
		B. 2 hours after injection			
156	26	35	195	15	3.4
118	67	57	114	14	2.7
187	92	73	139	18	17.0
374	280	195	183	28	16.7
410	78		135	11	26.0
248	59	54	168		15.4
Av. 249	100	83	156	17	
		C. 24 hours after injection			
110	142	128	53	41	3.3
115	125	115	33	11	3.8
Av. 113	134	122	43	26	
		D. 48 hours after injection			
98	105	103	51	18	17.0
101	110	112	32	17	19.2
102	115	97	40	17	18.5
288	298	304	68	51	12.3
Av. 147	157	154	48	26	

an extracellular phase of 33 per cent to cat heart and 11 per cent to cat striated muscle. The values for the P^{32} content of the intracellular inorganic phosphate of striated and cardiac muscles of the cats shown in tables 1 and 3 have been calculated in this manner from the data there presented.

Inspection of these data shows that no such relation exists between the P^{32}

contents of the intracellular inorganic phosphate and the PC or ATP as would be required by the diffusion theory. The data on the cardiac muscles are particularly striking: in all but the 24-hour animals the intracellular inorganic phosphate has a much lower P^{32} content than do the PC and ATP. At 24 hours, the situation is reversed: the intracellular inorganic phosphate has the highest P^{32} content, while the PC and ATP exceed the plasma phosphate in P^{32} content. The observed relations are opposite to those required by the diffusion theory, and are in full accord with the hypothesis presented above, that phosphate enters or leaves the cell only by formation or breakdown of an organic compound at the membrane. The data on the striated muscles of the cats, in table 1, also lead to this conclusion, as do those on the frog muscles. In the 24- and 48-hour frogs the P^{32} of the inorganic phosphate is slightly below that of the PC and ATP. This condition could exist only if the plasma P^{32} fell below that of the intracellular inorganic phosphate.

The relations between the various phosphorus compounds of muscle and plasma phosphate can be represented by the following diagram:



Additional evidence in favor of the view that phosphate enters the cell only by conversion to an organic compound at the membrane is found in the observation of Furchgott and Shorr (3) that in heart slices equilibrated with inorganic phosphate containing P^{32}, the intracellular inorganic phosphate and the PC have the same P^{32} content, at a level only one-fifth that of the medium. If the phosphate entered by diffusion, then at equilibrium the P^{32} content would be the same in the intracellular and extracellular phases.

With regard to the diffusion of phosphate through the capillary wall, the calculated values for the P^{32} content of the extracellular inorganic phosphate show that equilibrium is not established in one hour. On the assumption that all the P^{32} of the inorganic phosphate of cardiac or striated muscle is in the extracellular fluid, the P^{32} of this is between one-half and three-fourths as high as in the plasma P, at one hour after injection. Thus diffusion of phosphate through the capillary wall is appreciably slower than diffusion of sodium (6).

Granting that phosphate enters the cell only by conversion to an organic compound at the membrane, it becomes evident that PC and ATP exchange independently of each other at the membrane as well as with intracellular inorganic

phosphate. If this were not the case, i.e., if either one were the sole source of exchange at the membrane and the other exchanged only with intracellular inorganic phosphate, then the P^{32} content of the second one would be dependent on the P^{32} content of the intracellular inorganic phosphate. The data on the 4- and 24-hour animals show that this is not the case. It therefore appears to be a purely fortuitous circumstance that the turnover rates of PC and ATP are so close together.

The HMP does not undergo any interchange with intracellular inorganic phosphate or with PC or ATP. If such exchanges took place, then the P^{32} contents of the four intracellular compounds should become equal in time. The data show clearly that this is not the case.

Reverting now to the finding of Furchgott and Shorr (3) that the P^{32} content of the intracellular inorganic P and PC of heart slices at equilibrium is one-fifth that of the phosphate of the medium, the most logical explanation would seem to be that, for each molecule of creatine which becomes phosphorylated at the membrane, 4 react with intracellular inorganic phosphate. This ratio can, of course, be determined accurately only when the P^{32} content of the medium remains constant, as in experiments with slices, or by perfusion techniques. However, the present data on the intact heart indicate a ratio reasonably close to this.

Similarly, the 50 per cent turnover time can also be determined accurately only with a constant P^{32} level of the medium, but an approximation can be obtained in experiments on the intact animal from the time required for the P^{32} content of the substance in question to reach the maximum level. The data on the heart indicate a 50 per cent turnover time of about an hour for the PC and ATP, and appreciably longer times for the AA and HMP.

In the case of the striated muscle, it is possible from the present data to make only the roughest sort of approximation of the 50 per cent turnover time or of the ratio of molecules of creatine or AA phosphorylated at the membrane and with intracellular inorganic phosphate. The 50 per cent turnover time seems to be of the order of 4 hours; the ratio of phosphorylations at the membrane to intracellular phosphorylations is perhaps 1 to 50 or 1 to 100.

The turnover time of the HMP is extremely difficult to approximate. Comparisons of the P^{32} contents of striated muscle HMP and plasma P at 4 hours after the injection indicates that the 50 per cent turnover time of the HMP is of the order of weeks, rather than hours. In cardiac muscle the turnover is much more rapid.

Such rapid rates of breakdown and resynthesis of compounds in the resting metabolism of living tissue seem a good deal less startling at this date than they would have before the brilliant work of Schoenheimer on the rate of incorporation of heavy nitrogen into the tissue proteins.

The finding of such rapid interchanges of PC and ATP with inorganic phosphate in the resting metabolism of muscle offers some hope of reconciling the divergent views on the function of the phosphate compounds in contraction. In the formulation based on extract studies, the numerous phosphate interchanges have been directly connected with the formation of lactic acid, and it

has therefore been postulated that they were concerned with the formation of lactic acid in anaerobic contraction. The studies on the actual chemical changes in the contracting muscle, on the other hand, have found no evidence for the participation of the phosphate compounds in the formation of lactic acid. The present data indicate that if the phosphorylating glycolysis plays any part in the metabolism of muscle, it should be related to the resting metabolism, and not to the activity metabolism. This implies, of course, that the resting and activity metabolisms follow two radically different pathways. This is by no means the first indication of such a qualitative separation of the two types of metabolism. For contraction in the presence of oxygen, there is the finding of Stannard (9) that azide abolishes the excess oxygen consumption of the activity in concentrations which do not affect the resting oxygen consumption. For anaerobic contraction, there is the evidence (7) that the mechanism by which iodoacetic acid inhibits the formation of lactic acid in contraction is different from the one by which it inhibits the formation of this substance in extracts. This last point again brings attention to the possibility of a non-phosphorylating glycolysis in contraction, by way of methyl glyoxal. The iodacetate inhibition in this case might well be due to the destruction of reduced glutathione, the coenzyme of glyoxalase (5).

Granting that some of the reactions of the phosphorylating glycolysis may take place in the resting metabolism of muscle, it is evident that the entire cycle cannot be accepted without reservation. Participation of glucose-6-phosphate is ruled out by the finding that HMP does not interchange with PC, ATP or intracellular inorganic phosphate. However, there is no reason to rule out the possible formation of glucose-1-phosphate. If this were to undergo further reaction without being converted to the 6-phosphate, there would be no barrier to the acceptance of the phosphorylation cycle as the mechanism of aerobic glycolysis in the resting metabolism of muscle. There would then be no occasion to postulate that it is operating in contraction, irrespective of whether this takes place in the presence or absence of oxygen. Such a situation would still leave unsolved many problems in both fields. The function of phosphocreatine in the resting metabolism is apparently more important than the most recent developments of the phosphorylation cycle would indicate; furthermore, the mechanism of lactic acid formation in anaerobic contraction still remains to be elucidated.

The senior author is indebted to the Faculty Research Fund of the University of Michigan for a grant for the purchase of the Geiger-Müller counter, and to the trustees of the Horace H. Rackham Foundation for their support of the operation of the cyclotron. Thanks are due to Prof. J. M. Cork of the Department of Physics for the supply of radioactive phosphorus used in these experiments, and to Prof. O. S. Duffendack of the same department for his aid in the design of the Geiger-Müller tube.

SUMMARY AND CONCLUSIONS

1. The time course of the distribution of radioactive phosphorus in resting striated muscle has been studied in cats and frogs, and in the heart in cats.

2. The diffusion of phosphate through the capillary wall is relatively slow in comparison to other ions which have been studied. Equilibrium between plasma and extracellular fluid is not reached in one hour.

3. Phosphate enters the interior of the cell of striated or cardiac muscle only by being converted to an organic compound at the membrane, and leaves the cell only by the dephosphorylation of these compounds at the membrane.

4. The formation of phosphocreatine, adenosine triphosphate and hexosemonophosphate are independent processes, rather than being part of a cycle.

5. The phosphorylation of creatine and adenylic acid may take place either at the cell membrane or with inorganic phosphate present within the cell.

6. For each molecule of creatine or adenylic acid which is phosphorylated at the membrane, there are approximately 4 molecules phosphorylated intracellularly, in the heart, and a much larger number in striated muscle.

7. The hexosemonophosphate of striated or cardiac muscle does not interchange, directly or indirectly, with intracellular inorganic phosphate, phosphocreatine or adenosine triphosphate.

8. The turnover rate of phosphocreatine and adenosine triphosphate is independent of the amount of phosphate injected.

9. When large amounts of phosphate are injected there is a temporary accumulation of hexosemonophosphate on the cell membrane of striated muscle.

10. There is a rapid breakdown and resynthesis of phosphocreatine and adenosine triphosphate in the *resting* metabolism of striated and cardiac muscle.

11. There is evidence that some, but not all, of the reactions of the phosphorylating glycolysis that have been found in cell-free muscle extracts may take place in the *resting* metabolism, but not in the *activity* metabolism, of striated and cardiac muscle.

12. The chemical transformations associated with the contraction of striated and cardiac muscle are qualitatively different from those concerned with the resting metabolism.

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EFFECTS OF VISIBLE RADIATIONS UPON ALBINO RATS

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Received for publication July 20, 1942

Seasonal fluctuations in the weight of animal organs have been noted by a number of investigators. Brown, Pearce and Van Allen (2) maintained rabbits in environments of constant illumination, solar illumination and darkness and noted that those under constant illumination tended to have organs of relatively smaller weight than those of the controls and that the periodic fluctuations in the size of the organs were absent. Over a period of four years, Del Castillo and Pinto (3) observed a periodic fluctuation in the size of the testes and the prostates of rats. Following extensive work on the effect of light upon the sexual development of birds, Bissonnette (1) concluded that the progressive changes in the testes in the spring may be helped by the relative strength of red rays in the sunlight of that season and that the natural regression in summer and early autumn may be due to the relative excess of inhibiting or lethal green (and perhaps violet and ultraviolet rays) of this season. In 1939, Luce-Clausen and Brown (5) found that visible radiations promoted growth of rats, that the opening of the vagina and onset of oestrus were delayed in rats confined to darkness, and that the survival of young rats was higher under visible radiations. In 1941 Fiske (4) reported that female rats, kept under light from birth or from the twenty-first day of life, matured much earlier than females kept in darkness, that unusually long oestrus periods were characteristic of the former while frequent metoestrus occurred among the latter, and that the pituitaries, ovaries and uteri of 77 day old rats, kept under light for 8 weeks, were heavier than those of litter mates kept in darkness.

In the present investigation an attempt has been made to ascertain whether some portions of the visible spectrum are more effective than others upon growth, activity, basal metabolism, reproduction and survival of litters of albino rats.

METHODS. Cages were set up on shelves 7 feet away from and facing two 100 watt Mazda electric bulbs. The front and top of these cages consisted of $\frac{3}{8}$ inch mesh galvanized wire while the sides and back were made of galvanized sheeting. In front of each cage was placed a cellophane filter, either black, colorless, red, orange, yellow, green, blue or violet. With the exception of the black, these filters consisted of a single thickness and are described by the manufacturers, Canadian Industries Limited, as no. 300 plain transparent, red, tango, amber, light blue and violet respectively. The percentage of light transmitted and the region of transmission for each of the colored filters was determined[1] and is shown in figure 1. The black filters consisted of a heavy sheet of

[1] This determination was made possible through the co-operation of the Department of Physics and was carried out by Thomas Collins.

kraft paper covered on both sides with black cellophane. To eliminate any seasonal influence, the temperature of the rat room was thermostatically controlled at 70°F. and the period of illumination electrically controlled at 10 hours per day.

Three series of Wistar albino rats have been used in this work. In the first series, an exploratory run begun in 1939, 8 males and 8 females at 5 weeks of age were placed in pairs behind the 8 filters described above and carried for 190 days. In the second series, begun in 1940, 27 rats at 3 weeks of age were avail-

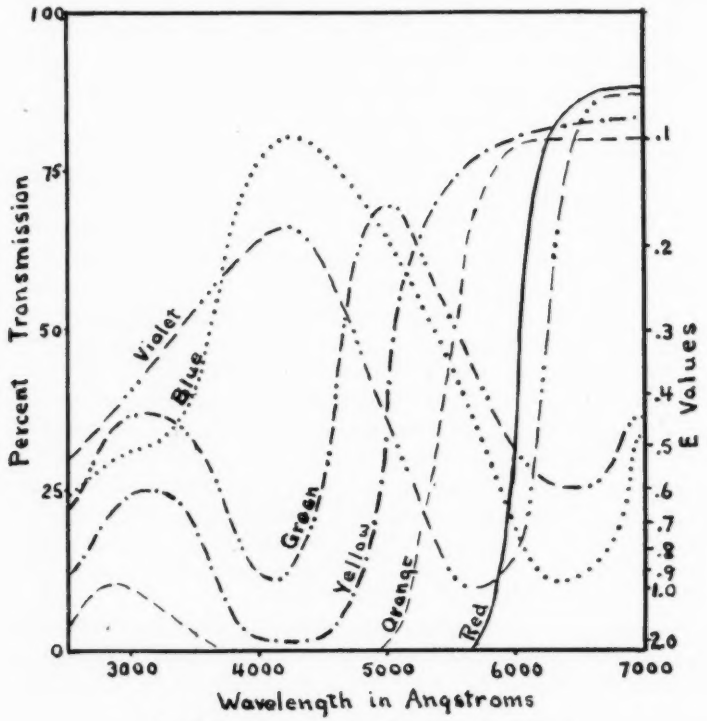

Fig. 1. Light transmitted by cellophane filters.

able. Behind each of the 8 filters were placed 1 male and 2 females. This left 3 more females which were placed behind the red, blue and violet filters. A few weeks later an error in sexing the rats was discovered. Behind the colorless filter there were 2 males and 1 female. This second series was carried for 212 days. The third series, begun in 1941, consisted of the F_1 generation of the second series with the exception of those behind the blue filter. Since no litters had been born behind the blue filter, rats for this filter in the third series had to be drawn from new stock. This series was started when the rats were 3 weeks old. Behind each filter were placed 1 male and 2 females. Two cases of incorrect sex-

ing were later discovered in this series, behind the colorless and the red filters where in each there were 2 males and 1 female. After carrying this third series for 180 days, the filters were changed so that rats, previously behind colorless or colored filters were placed behind black filters while those behind black filters were placed behind blue filters. Following this change the series was carried another 210 days; at the end of this time the original filters were restored and the series completed by the end of 21 days more.

Each adult rat was given three Purina Fox Chow checkers per day. While nursing its litter each female was given two additional checkers per day. Occasionally all rats received supplements of carrots and green leafy vegetables.

At the time of casting of a litter the other rats in that cage were removed to another behind a similar filter. When the litter was 3 weeks old, the mother was returned to her original group.

Growth was determined from weight increases. All rats were weighed once a week between the ages of 21 and 70 days. Thereafter the males were weighed once a week while the females were weighed twice a week. Each litter was handled as little as possible and consequently was weighed as a whole at birth and at 3 weeks of age.

Activity measurements were attempted with two pieces of apparatus constructed for the purpose. In one of these, initial activity (the first 90 sec.) was sought, while in the other activity over a 24 hour period was investigated. In both cases the tests were carried out 18 to 24 hours after feeding.

The apparatus used for determining initial activity consisted of a rubber diaphragm stretched tightly over a four-inch funnel by means of twelve small battery clamps anchored to an iron retort ring at a lower level. Fitting around the edge of the funnel and extending upward 14 inches a cardboard cone served to retain the rat on the rubber diaphragm. The stem of the funnel was connected by rubber tubing to a tambour. The thin rubber membrane on the tambour was fastened securely but not stretched, in order to permit greater movement of the writing lever attached which recorded on a smoked drum revolving once in 90 seconds. Because of the friction of the writing lever on the drum and the elasticity of the air in the apparatus, it was found that greater movements were recorded on the drum when the air in the apparatus was replaced by water. Each rat was dropped head first from just above the cardboard cone and the movements recorded. The preserved records were enlarged by projection on a translucent screen and the length of the activity line minus the base line determined.

The second activity apparatus consisted of a chamber 24 in. wide, 8 in. deep and 30 in. high. It was divided into an upper, middle and lower chamber by three boards constituting the floors of these chambers. These boards were cut across the middle and hinged there. The outer ends of each of these were slightly raised by a light spring. As a rat moved out towards the end of one of these boards, the outer end descended 1 cm. and in so doing passed an electrical contact. This caused a signal magnet to record the movement and also caused an electro-

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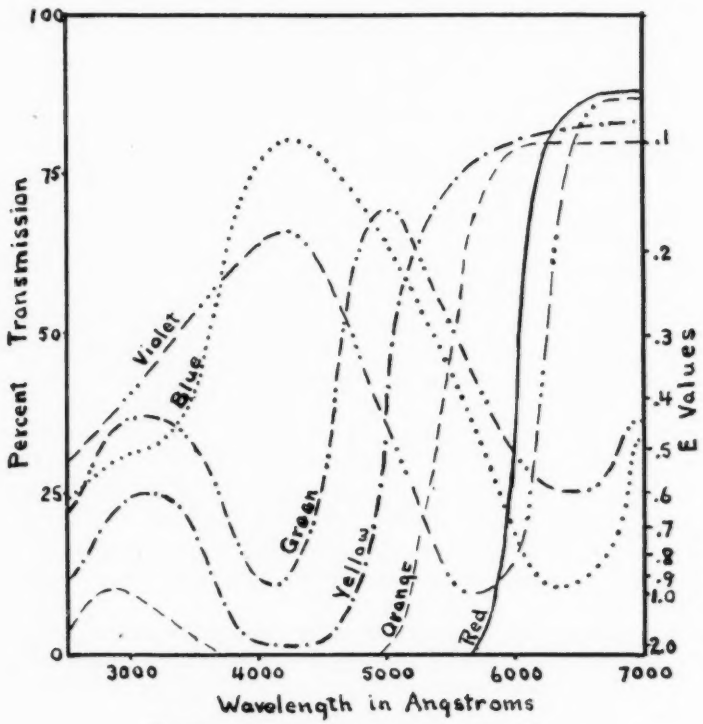

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The second activity apparatus consisted of a chamber 24 in. wide, 8 in. deep and 30 in. high. It was divided into an upper, middle and lower chamber by three boards constituting the floors of these chambers. These boards were cut across the middle and hinged there. The outer ends of each of these were slightly raised by a light spring. As a rat moved out towards the end of one of these boards, the outer end descended 1 cm. and in so doing passed an electrical contact. This caused a signal magnet to record the movement and also caused an electro-

magnet to rotate the smoked double drum about 2 mm. When the rat moved back towards the center of that floor another contact was made as the outer end of the board rose and this movement was recorded. In like manner movements of the other boards at each level moved signal magnets and were recorded. A sloping runway permitted the rat to move from one level to the next. A seventh signal magnet attached to a clock recorded one hour intervals.

Basal metabolism was determined in an apparatus constructed, with a few modifications, on the plan of Schwabe and Griffith (6). The chief modification was the manner of drawing oxygen through the apparatus and of removing carbon dioxide. By lowering and raising a reservoir Schwabe and Griffith caused the air in the rat chamber to be brought in contact with a standardized barium hydroxide solution for removal of the carbon dioxide and the return of the residual air to the rat chamber. In the present investigation the rat chamber consisted of a flat-bottomed cylindrical glass vessel 9 in. in diameter and 3 in. deep. This was inverted in a narrow circular mercury trough on a varnished copper plate. Coming through the floor of the chamber was a sealed-in copper tube connecting the chamber with the rest of the apparatus and delivering oxygen to the chamber at a rate equal to the rate at which it was consumed by the rat. The carbon dioxide and water vapor set free by the rat were absorbed by soda-lime placed between two pieces of copper gauze to form a wall $2\frac{1}{2}$ in. high and $\frac{3}{4}$ in. thick extending around the chamber just inside the glass wall. The wire gauze was held in place by small wooden posts $2\frac{1}{2}$ in. long nailed in an upright position to a thin circular wooden disc which fitted inside the chamber. In recording the rate at which oxygen was utilized by the rat, a cylindrical glass float was substituted for the paraffined cork used by Schwabe and Griffith. This eliminated small fluctuations in the oxygen curve which appeared with the formation and collapse of each oxygen bubble due to the oversensitiveness of the cork float. The glass float substituted, while not registering these, was quite sensitive to any change in activity of the rat, as seen by an increase in the slope of the oxygen curve with the slightest movements of the rat. Construction and testing of this apparatus was completed in April 1942. This has permitted its utilization in carrying out basal metabolism determinations in the third series of rats under two sets of conditions. In the first of these, the rats, after 180 days behind the 8 types of filters, had been switched to the black or the blue filters, as previously described, and were in the last month of this 210 day period. The second set of conditions covers the final 21 day period of this series during which the rats were behind their original filters.

Results and discussion. *A. Growth.* After studying the growth curves of the rats in the three series, it became evident that there was no consistent difference, either in the weights of the rats at any particular age or in the rates of growth, that could be attributed to the filters used.

B. Activity. Neither activity apparatus was completed in time to be used with the first series of rats. In the second series, initial activity graphs were obtained but no satisfactory method developed for determining quantitatively the relative activities expressed by these graphs. However, inspection of these

graphs definitely placed the rats behind the red and the black filters as the most active, those behind the orange the least active, and the others in an intermediate position. In the third series, the relative initial activities were determined quantitatively and are summarized in table 1. With the exception of the position of the rats behind the yellow filter, which have moved from an intermediate to the top position, the order of activity remains practically the same as the second series.

Rats placed in the second activity apparatus for the 24-hour test showed activity only in the first 4 hours and the greater part of this in the first 2 hours.

TABLE 1

Initial activity

	Filter							
	Yellow	Red	Black	Green	Colorless	Blue	Violet	Orange
Activity	102.3	72.4	66.8	57.3	43.5	40.0	37.2	31.5

TABLE 2

Basal metabolic rates

	Filters							
210-day period								
Original color	Yellow	Red	Black	Violet	Orange	Blue	Colorless	Green
to	Black	Black	Blue	Black	Black	Black	Black	Black
B. M. R. (a)	52.9	44.8	43.8	43.4	43.0	41.2	40.1	40.0
21-day period								
Restored to	Yellow	Blue	Black	Violet	Red	Orange	Colorless	Green
from	Black	Black	Blue	Black	Black	Black	Black	Black
B. M. R. (b)	41.4	39.5	38.7	37.1	36.4	35.8	32.2	30.6
Original color	Yellow	Green	Red	Colorless	Orange	Violet	Black	Blue
Drop in B. M. R. (a − b)	11.5	9.4	8.4	7.9	7.2	6.1	5.1	1.7

These tests, therefore, were reduced to 4 hours. However, the variation in response of the same rat on repeated tests on different days was too great to make use of such tests for measuring activity.

C. Basal metabolism. The basal metabolic rate for each rat was determined on three separate occasions during the last month of the 210 day period. From these the average for each rat was determined and in turn an average worked out for all rats getting the same radiation. It is this last group of figures which is shown in table 2. In like manner for the 21 day period after returning the rats to their original filters, three determinations were made with each rat and an average taken. Then from these individual averages, averages for all

rats getting the same radiation were determined. In calculating the surface area of the rat, Rubner's formula was used. The basal metabolic rates are expressed as kilogram-calories per square meter per hour.

It will be recalled from table 1 that rats behind the yellow filter were the most active. From table 2, this same group has the highest basal metabolic rate. Further, all groups showed a lower B. M. R. when returned to their original filters for 3 weeks from black filters, or from behind blue in the case of original blacks. This drop in B. M. R. was greatest for the yellow filter followed closely by green, red and colorless and was least in restoration of blue from black.

D. Reproduction. In the first series of rats litters were cast behind all filters except the blue. Autopsies at the end of the test period, 190 days, revealed no developing feti in the rats behind the blue filter. Toward the litters receiving radiations through the different filters there appeared to be, in general, less care given by the mother and a greater cannibalistic tendency than usual and this occurred independently of the size of the litters.

In the second series of rats these points were investigated further. At the completion of this series, 212 days, all females, with the exception of those behind the blue filter, had cast litters. At this stage, two of the three females behind the blue filters were given three intramuscular injections of 1 cc. each, at 3-day intervals, of oestradiol benzoate in sesame oil (0.2 mgm per cc.).[2] At the same time the male was given like injections of testosterone propionate in sesame oil (5 mgm per cc.).[2] By the end of a further 90 days there still were no litters cast behind the blue filter.

Since this was the second group of rats which had been raised behind the blue filter and had failed to cast a litter, it seemed unlikely that chance sterility was the cause. However, to be sure of this a third group was started behind the blue filter and placed in the third series. At an age of 180 days this third group of rats behind the blue filter had cast no litters. At this stage, switching the blue filter for a black over an additional 210-day period, did not alter the situation as far as litters were concerned.

In both the second and third series of rats it will be seen by reference to table 3 that rats raised behind the black filter stood well up in the list with regard to the number of offspring per rat but were bettered by rats behind the yellow filter. On the other hand, reproduction behind the colorless filter appeared to have been repressed.

In the last column of table 3 the colors given refer to the original filters. It will be recalled that these were switched so that rats behind black were given blue and all other filters were replaced by black. Further, with the exception of rats behind the original blue filter, all in the third series belong to the F_1 generation of the rats in the second series. It should also be noted that the length of the first part of the third series is a month shorter than the balance of that series or the full second series. Keeping these points in mind, it would appear that rats behind the yellow filter in both series had the same degree of fertility and this

[2] We are indebted to Ciba Co. of Montreal for these preparations.

was not affected by the switch of filters from yellow to black. Rats behind the black filter showed approximately the same fertility in the second series and first part of the third but with the switch to blue in the second part of the third series their fertility dropped about 25 per cent. Rats behind the violet filter in both series had about the same fertility until the filter was switched to black when the fertility dropped more than 50 per cent. A similar story holds for green with an even greater drop in fertility after switching to black. Rats behind the red filter showed a sharp decrease in fertility in the F_1 generation

TABLE 3

Number of offspring per rat

SECOND SERIES		THIRD SERIES			
212 days		First 180 days		Next 210 days (filters switched)	
Yellow	24.0	Yellow	17.0	Yellow	23.5
Red	23.7	Black	16.5	Black	18.5
Violet	22.0	Violet	13.0	Violet	9.5
Orange	22.0	Green	8.0	Green	3.0
Black	20.5	Red	3.0	Red	0
Green	13.5	Colorless	2.0	Colorless	0
Colorless	4.0	Orange	0	Orange	0
Blue	0	Blue	0	Blue	0

TABLE 4

Percentage survival to 21 days in litters

SECOND SERIES			THIRD SERIES				
212 days			First 180 days			Next 210 days	(Filters switched)
Black	5/41	83.0%	Yellow	5/34	88.2%	Green 1/3	100.0%
Colorless	1/4	75.0	Black	4/33	81.8	Yellow 7/47	97.9
Violet	9/66	74.3	Violet	3/26	80.8	Violet 4/19	89.5
Green	5/27	63.0	Green	3/16	68.8	Black 5/39	45.9
Red	7/71	55.6	Colorless	2/4	50.0		
Orange	5/44	40.9	Red	1/3	0		
Yellow	6/48	39.6					

with no litters cast in the last 210 days. While rats behind the orange filter in the parent generation produced well, no litters were cast in the F_1 generation.

This decreasing fertility exhibited in the third series and particularly in the 210-day period can not be due entirely to the increasing age of the rats for at the end of the 210-day period they were only 390 days old. Further, if age were the controlling factor, the effects would be more evenly distributed over all the test animals. It would seem that radiation is a factor, possibly the prime one in this case, since behind black filters fertility of the rats was maintained while behind all other filters used, except yellow, fertility decreased at different rates and to

different degrees. After 180 days' radiation, this decreasing fertility was not halted by placing the rats behind black filters.

E. Survival of litters. The effect of using the different filters on the survival of the litters to 21 days is shown in table 4.

As in table 3, the colors in the third column refer to the original filters used. Where no litters were born, the filters are not mentioned. The number of litters cast and the total number of offspring in these litters is respectively indicated by the numerators and denominators of the fractions placed immediately after the color of each filter. In four cases the survival values are based on a rather limited number of offspring and should be considered with this in mind. The cases referred to are the colorless in the second series, the colorless and the red in the first part of the third series, and the green in the last part of the third series.

Consideration of the survival values shown for the second series and first part of the third places the black filter at the top with a value of 82.4 per cent, followed by violet (77.5), green (65.9), yellow (63.9), colorless (62.5), orange (40.9) and red (27.8). Whether this order is coincidental or significant, it is nteresting to note that it is the same as in the solar spectrum. The second part of the third series would seem to further establish the position of the black filter toward favoring the survival of litters, since switching green, yellow and violet to black improved survival values while switching black to blue considerably owered the chance of survival.

SUMMARY

Growth, initial activity, basal metabolism and reproduction of albino rats and the survival of their litters behind black, colorless, red, orange, yellow, green, blue and violet filters have been compared.

No consistent difference either in the weights of rats at any particular age or in their rates of growth was found which could be attributed to the color of the filter used.

Initial activity was influenced by the color of the filter used, being greatest under yellow, followed by red and black and least under orange.

Basal metabolic rates were highest under the yellow filter and lowest under the green.

The number of offspring per rat was highest under the yellow filter throughout the experiment while under the blue filter reproduction was inhibited from the start. The other filters exhibited degrees of inhibition.

Survival of litters to 21 days was highest behind the black filter and lowest behind the red.

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THE RELATIONSHIP BETWEEN MONOCHROMATIC LIGHT AND PUPIL DIAMETER. THE LOW INTENSITY VISIBILITY CURVE AS MEASURED BY PUPILLARY MEASUREMENTS

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Received for publication March 16, 1942

It has been known for some time that there is a considerable degree of correspondence between pupillomotor and sensory effects of stimulation of the retina by light. Reeves in 1918 showed that during dark adaptation, after previous exposure of the eye to a bright light, the curve for pupillary dilatation closely parallels that for increasing retinal sensitivity. Others subsequently found this to be true (Crawford, 1936; Brown and Page, 1939, both on humans; Gullberg, Olmsted and Wagman, 1938, on rabbits).

This correspondence is further seen in studying the effect of different wave lengths of light on the size of the pupil. Although these studies have been few, they do point to the fact that the visibility curves of the eye obtained by measuring the variation in pupil diameter in the different wave lengths are comparable to those obtained by the more usual subjective means. Laurens (1923), as Sachs (1892) and Abelsdorff (1900) had done earlier, determined the visibility curves of the human eye using the pupil diameter as a measure. Hess (1913, 1915) studied the effects of different spectral lights and of the lights transmitted by colored glasses on the pupil reactions of cats and rabbits. He found that the maximum pupil constriction for these animals in the light adapted state was at about 550 mμ and in the dark adapted state was at about 505 mμ. Laurens (1923) determined the visibility curves of both the pigeon and the alligator by means of pupillary measurements. In the pigeon the maximum effect in the light adapted eye was at 564.1 mμ, and in the dark adapted eye at 544.2 mμ. In the alligator eye these maxima were at 544.2 mμ and 514.2 mμ respectively.

Hecht and Pirenne (1940) investigated the retinal sensitivity of the nocturnal long-eared owl, using the pupillary size as a measure of sensitivity. They measured the relative effectiveness of different wave lengths of light in causing a constant amount of pupil contraction, and found that the spectral visibility curve for the owl is the same as the human visibility curve at low light intensities, with a maximum at about 515 mμ.

These studies lead to the conclusion that pupillomotor action depends upon two receptors, just as does retinal sensitivity; namely, the rods and cones. This view is supported by the results of other studies, the majority of which point to the fact that both receptors play a part in the regulation of the size of the pupil, although the effect of the cones predominates. (cf. Laurens, 1923; Ferree, Rand and Harris, 1933, and Brown and Page, 1939, for reviews on this subject.)

Brown and Page (1939) concluded from a study of dilatation of the pupil in darkness that the pupil size relative to light stimulation is under the control of fibers activated only by cones in the central portion of the retina. These two workers show that the course of pupillary dilatation as measured by them, by Reeves (1918) and by Crawford (1936), bears a strong relationship to foveal dark adaptation as measured by Hecht (1921a) and by Hecht, Haig and Chase (1937). Pupil dilatation, just as foveal dark adaptation, is almost complete in about one minute, and practically all completed in five or six minutes, and fully complete in ten minutes at the most. Brown and Page believe that the Purkinje effect would result from transmission of excitation from the rods within or outside the macula to optic nerve fibers leading off from cones and controlling pupillary constriction, and therefore that the rods have no influence on the movements of the pupil.

An attempt is here made to measure the low intensity human visibility curve using the objective measure of pupillary size. If the rods have any effect on the pupillary response, we should be able in this manner to detect a rod influence if it exists.

The work which has been done on spectral sensibility of the human eye using the pupil as a measure has been slight and, in general, unsatisfactory. Although the work of Laurens (1923) remains the best of the studies done on this particular problem (cf. Sachs, 1892, and Abelsdorff, 1900) there is a very significant objection to it. Laurens determined the amount of pupillary contraction in the pigeon and alligator as well as in the human caused by different wave lengths of equal energy. According to the classical accepted definition of a spectral luminosity curve, this latter study is not accurate. Such a curve, to be acceptable and comparable to other investigations, must record the reciprocal of the relative energy required in different parts of the spectrum to produce the same physiological effect (Hecht, 1928; Graham and Hartline, 1935; Hecht and Pirenne, 1940).

Method. Infrared photography is the most suitable method for accurately measuring the diameter of the pupil in virtual darkness as well as under any condition of light adaptation. Infrared light does not influence the size of the pupil (Crawford, 1936; Gullberg, Olmsted and Wagman, 1938; Brown and Page, 1939). This method can also be satisfactorily used when a beam of colored or white light is directed into the eye.

The apparatus devised for these experiments consisted essentially of three distinct parts:

1. An instrument for adapting the eye to a spot of colored light of any intensity within the necessary range, and having any diameter up to about 16.6 degrees visual angle (fig. 1).
2. A camera and auxiliary apparatus for photographing the pupil (figs. 1 and 2).
3. An infrared source of light which is used to illuminate the eye when photographing.

The adapting instrument used fulfills all the specifications needed to pursue work on both light and dark adaptation (Hecht and Shlaer, 1938). The intensity, the color, and the duration of the preadapting light may be varied to suit any of

the conditions of the experiment. Provisions are made to vary the area and retinal location of the light in the eye. This instrument can also be used for subjectively measuring the visual threshold after any period of dark adaptation.

The instrument is shown diagrammatically in figure 1. The source of light is the circular ground glass window, *G*, 20 mm. in diameter, illuminated by a 6 volts, 18 amperes, coiled filament projection bulb, *T*, which has been seasoned.

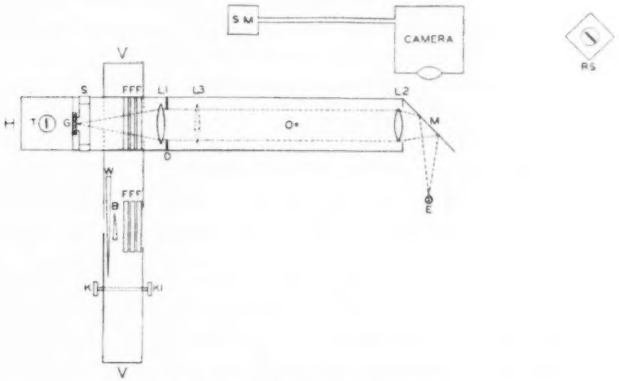

Fig. 1. Explanation in text

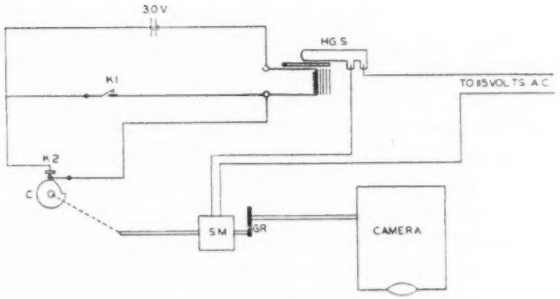

Fig. 2. Explanation in text

The lamp burned at 6.2 volts and its operating characteristics were checked at frequent intervals.

The lens *L 1*, 60 mm. in diameter, is placed at its principal focal length of 12 cm. from the secondary source of ground glass *G*. The beam of approximately parallel light is picked up by another lens, *L 2*, having a diameter of 48 mm. and a focal length of 15 cm. It is then reflected through an angle of 90 degrees by the partially aluminum-sputtered mirror, *M*. Thus an image of the source *G* is produced in the plane of the pupil of the eye at *E*, and the lens *L 2* appears uniformly illuminated and seems to be placed directly in front of the observer.

Near the lens *L 2* is a groove, *D*, which is made to hold metal diaphragms of varying sizes in order to control the beam of light. The maximum visual angle obtainable is 16.6 degrees with a diaphragm of 44 mm. opening.

L 3 is a lens of 60 mm. in diameter and 45 cm. focal length which can be substituted for *L 2*. When it is in place without *L 2* and when used with a maximum size diaphragm at *D*, an image of the source is produced having a visual angle of 5.7 degrees.

A Compur shutter, *S*, is placed in front of the light source and is used to regulate the exposure time from $\frac{1}{50}$ of a second up to minutes of exposure.

V is a movable mount to carry colored and neutral filters, *F*, neutral wedge, *W*, and balancing wedge, *B*. This carrier can be displaced in such a manner as to permit utilization of full intensity of light or of the different possible combinations of neutral filters and wedge to give an intensity range of from 1 to 10^{-12}.

Six Wratten neutral filters were used transmitting from $\frac{1}{2}$ to $\frac{1}{10\,000}$ of the incident light. The wedge is 15 cm. long and covers an intensity range of from 1 to 1000. It is used in conjunction with a balancing wedge so as to provide a uniform field.

The neutral wedge is moved by a rack and pinion, and has a scaled dial, gear connected so as to define the position of the wedge. To vary the spectral characteristics of the light the special set of Wratten Monochromatic Filters was used. This set has been extensively used in objective studies where the high intensities required were not easily attained with a spectroscope (Hecht, 1921b; Crozier, 1924; Hecht, 1928; Grundfest, 1932a, b; Graham and Riggs, 1935; Graham and Hartline, 1935; Hecht and Pirenne, 1940).

The eye when in a position to be adapted and photographed was fixated on a target, *O*, which was placed 10.5 cm. in front of lens *L 2*, giving a virtual image 45 cm. from the eye. The fixation point was a May Ophthalmoscope bulb blackened except for a small point which provided a red window. The brightness of this small red dot was controlled by a rheostat so as to keep its intensity at or slightly above the threshold of the subject.

Since 45 cm. is within the accommodation power of the emmetropic eye, the question arises as to whether the pupil diameter is influenced by near accommodation, and if so, to what extent. Many subjects used here were myopic with a refractive error of -2.25 D or greater, so that in fixating at 45 cm. they relaxed accommodation, and their pupil diameter was not influenced. Luckiesh and Moss (1934) found that for the emmetropic eye, at a brightness level of 0.1 milli-lambert, the pupil size remained at its maximum at fixational distances of 60 cm. and beyond. At 45 cm. the pupil reached a diameter which was only slightly less than the maximum.

In any event this was kept a constant factor in these studies. Attempts were made to learn whether an increased pupillary size could be obtained by relaxing accommodation in the dark adapted eye. The effect was found to be negligible.

The camera used to photograph the pupil of the eye was the model J, Debrie 35 mm. motion picture camera. It was provided with a Zeiss Biotar 50 mm. focal length, F 1.4, mounted so as to permit photography at a distance of 15 cm.

The camera was driven by a rod mechanism geared to a synchronous motor, *SM*, in figure 2. The gear ratio was 1 to 10 (*GR*) and when the camera was set to operate at one film per revolution, the complete cycle for taking a photograph was 4.2 seconds. A hand key, *K 1*, initiates the camera cycle by operating a mercury switch, *HG.S*. The rotation of the camera drive actuates a cam mechanism, *C*, which closes a parallel electrical circuit *K 2* to continue rotation until a position is reached where the photograph has been taken and the camera is arrested in preparation for the next cycle.

The exposure time is varied by the opening or closing of the angular aperture of the camera shutter. With Eastman infrared film it was found that a lightly sputtered mirror required an angular exposure setting of the order of $\frac{1}{8}$ of a second.

The infrared source of light shown as *RS* in figure 1 has been fully described elsewhere (Wagman, 1937: M.A. Thesis, Univ. of Calif. Library, Berkeley: Gullberg, Olmsted and Wagman, 1938). A 30-volt, 30 ampere motion picture projection lamp was used to obtain a concentrated beam of light emitting a large proportion of infrared energy. The tungsten lamp is used with a spherical reflector and two B. and L. Cinephor aspheric condensing lenses. A Wratten no. 87 filter was mounted 10.5 inches in front of the lenses, the whole unit being enclosed in a light-tight box provided with adequate baffles for good air circulation. The 900 watt lamp was controlled by a carbon plate rheostat and a Weston wattmeter. When adjusted to operate at 400 watts such a lamp and filter contain only a small component of visible red in addition to infrared. The eye was illuminated at an angle of about 45 degrees and it was found that this residual visible red had no measurable effect upon the pupil. The subject's head was held in position by a chin rest used in conjunction with a rest for the cheek. When the subject's eye was fixated above the target, the beam of light from the adapting instrument focused immediately upon the pupil which was also in focus for the camera. The eye could thus remain in a constant position during the entire length of the experiment. The adaptometer was well shielded by a wooden hood and since the infrared source is essentially light-tight in the visible range, the experiments were performed in complete darkness. The experimenter used a small flashlight provided with a red bulb to read instruments and check positions.

The time interval in the course of pupillary dilatation was checked either by observing a watch or using a signal magnet connected in series with key, *K 1*, and a time trace on a kymograph drum.

At the beginning of each photographic series a millimeter scale was placed in the plane equivalent to the pupil position and photographed. This provided a reference scale for the measurement of pupil size. The films were developed in Eastman "D-19" developer to a considerable degree of contrast. These negatives used with a "Brinell" measuring microscope gave pupil diameter with an accuracy of a fraction of a millimeter.

Calibrations. The brightnesses of white light from the adapting instrument obtained with and without each of the neutral filters, and for fifteen points on the neutral wedge as well, were measured at the plane of the pupil where a piece

of white blotting paper was placed. The reflectance of this paper for white light had been accurately calibrated by the Department of Mechanical Engineering. The brightness on the blotting paper surface of each particular light intensity was measured by means of a Luckiesh and Taylor Brightness Meter placed three feet away from this surface. After accounting for the reflectance loss on the blotting paper surface, the apparent brightness at that point was obtained in foot-lamberts. From them the brightnesses given by any combination of filters and wedge could be determined.

The Wratten Monochromatic filters were calibrated by an indirect method which has been used successfully by others. In addition to its being quite accurate, the method eliminates the effect of the infrared radiations which each of these filters transmit (Hecht, 1928; Grundfest, 1932a, b; Graham and Hartline, 1935; Hecht and Pirenne, 1940).

The method consisted first in measuring the percentage transmission of each of the filters for every ten milli-microns between 400 and 700 milli-microns,[1] and the relative energy distribution in the spectrum of the 108 watt lamp while it was in its usual place in the adapting apparatus. The latter measurement was made by determining the color temperature of the lamp in conjunction with the ground glass, the lenses, and the partially aluminated mirrors as they are set in the instrument. The color temperature was found to be 2980 degrees Kelvin[2].

The relative energy of the lamp at any wave length multiplied by the transmission of a filter at that wave length gives the amount of energy transmitted by the filter at that particular wave length. For each filter the energy transmission which was obtained for every ten milli-microns in the visible part of the spectrum only was plotted against wave length. The total relative energy transmitted by each filter was determined by measuring the area under its transmission curve by means of a planimeter.

The wave length which divides the curve into two equal areas was then determined planimetrically. This wave length corresponds to the center of the energy transmitted by each filter in conjunction with the lamp in the system, and is the wave length used in dealing with the results obtained. The information gathered by the above methods is given in table 1.

The calibrations obtained by the indirect method described were checked roughly, by means of both a thermopile and Weston photronic cell, each used with a sensitive galvanometer. The results obtained by both these latter methods agreed well with those shown in table 1.

The procedure used in studying the effect of white light on pupil diameter described by Wagman and Nathanson (1942) was used here as well. The effect of each of the seven wave lengths was measured at nine different points covering a range of from about five log relative energy units (at 688 mμ) to about eight

[1] We thank Dr. G. Mackinney of the Division of Fruit Products in the College of Agriculture for allowing us to use the Bausch and Lomb spectrophotometer and for his help in determining these transmission values.

[2] These measurements were kindly made by Mr. A. Collins of the Department of Mechanical Engineering on the campus.

log relative energy units (at 450 mμ). A curve relating the log relative energy for each wave length to pupil diameter in millimeters was drawn.

RESULTS. The data obtained on five subjects from the seven wave lengths are summarized in table 2. The pupil diameters represent averages for all subjects of all determinations taken at 10, 20, 30, 45 and 60 seconds after a particular light was thrown in the eye. The light remained 16.6 degrees visual angle throughout the experiments.

When pupil size is plotted against log intensity for each wave length, similar curves are obtained possessing a characteristic sigmoid shape, for white light and for lights of different wave lengths. Average values are used because there are slight differences from reading to reading. The differences are to be accounted for by the fact that not only are there variations in pupil size from individual to individual, but also in the same individual from day to day (Reeves, 1918; Laurens, 1923; Crawford, 1936). Since the measurements on each subject were made on different days over a period of several weeks, such variations are to be expected.

TABLE 1

Relative energies and central wave lengths of each of the Wratten monochromatic filters

FILTER	CENTRAL WAVE LENGTH	RELATIVE ENERGY
	milli-microns	
70	688	13.333
71A	656	6.610
72	613	1.344
73	581	3.236
74	532	1.687
75	491	1.703
76	450	1.000

The first measurement at each wave length was always the same pupil size as after a 20-minute stay in complete darkness. The first point for wave lengths 688, 656 and 613 milli-microns is an average for all the threshold values determined subjectively. No color could be detected at these intensities by the dark adapted eye. At all other wave lengths, the first point is at an intensity which also does not give a color sensation to the dark adapted eye, but which is slightly above the threshold value for these lights. The pupil diameter at these points was always the same as it was after 20 minutes of dark adaptation.

If all seven curves plotted from the data in table 2 are drawn to the same scale, it is possible to get a picture of the relative stimulating values of each wave length by determining the relative energy for each wave length necessary to cause a constant amount of pupillary constriction. By so doing it is found that 491 milli-microns is the most effective wave length. If all curves are superimposed it is found that the one for this wave length is farthest to the left. In other words, less energy is required to cause a pupillary constriction of, say, 0.5 mm. at this wave length than at any other. A wave length of 532 milli-microns

is slightly less effective in the dark adapted state. The other wave lengths in order of their decreasing effectiveness are: 450, 588, 613, 656 and 688 milli-microns. From a close inspection of the seven log-energy response curves obtained from the data of table 2, it can be seen that these curves maintain, on the whole, their relative positions throughout. There is no indication that the visibility curve, as determined here for a dark adapted eye, ever shows a cone response with a maximum visibility at about 580 milli-microns (Gibson and Tyndall, 1923; Sloan, 1928).

From this information the visibility curve may be determined. Visibility may be defined as the reciprocal of the relative energy necessary, at a given wave length, to produce a given constant physiological response. The visibility curve may be obtained from the various response-log relative energy curves by using the method described by Hecht (1928). One can read off from the curves the abscissa values corresponding to a given response on the ordinate. The reciprocals of these energy values give the relative stimulating capacities of the different

TABLE 2

Pupillary diameters of the human subject at different wavelengths of light at various relative energies

688 MILLIMICRONS		656 MILLIMICRONS		613 MILLIMICRONS		581 MILLICRONS		532 MILLIMICRONS		491 MILLIMICRONS		450 MILLIMICRONS	
Log relative energy	Average pupil diameter	Log relative energy	Average pupil diameter	Log relative energy	Average pupil diameters	Log relative energy	Average pupil diameters	Log relative energy	Average pupil diameters	Log relative energy	Average pupil diameters	Log relative energy	Average pupil diameters
$\bar{2}.4617$	6.2	$\bar{2}.8888$	6.6	$\bar{4}.5675$	6.8	$\bar{4}.9735$	6.9	5.2564	6.7	5.2523	6.8	5.4835	6.5
$\bar{1}.9890$	6.2	$\bar{2}.2937$	6.5	$\bar{4}.1332$	6.9	$\bar{3}.7516$	6.7	4.0345	6.6	$\bar{4}.0304$	6.2	$\bar{4}.2616$	6.4
0.2219	5.8	0.5266	5.8	$\bar{2}.9855$	6.8	$\bar{2}.6039$	6.6	$\bar{2}.8868$	6.1	$\bar{2}.8827$	6.3	$\bar{3}.1139$	6.4
1.2213	5.0	0.9066	5.1	$\bar{1}.2184$	5.9	0.8368	5.8	$\bar{1}.1197$	5.2	$\bar{1}.1156$	5.4	$\bar{1}.3468$	5.5
2.1377	4.5	1.8330	4.8	0.2148	5.5	0.5964	5.0	0.3135	4.4	0.3176	4.2	0.0864	4.5
2.6381	4.4	2.3334	4.4	1.1412	4.7	1.5228	4.6	1.2399	4.0	1.2440	4.1	1.0128	4.0
2.8330	4.0	2.5283	4.1	1.6416	4.4	2.0232	4.0	1.7403	3.6	1.7444	3.7	1.5132	3.8
3.1249	4.0	2.8202	3.6	1.8365	4.1	2.2181	3.8	1.9352	3.5	1.9393	3.5	1.7081	3.5
				2.1284	3.9	2.5100	3.6	2.2271	3.1	2.2312	3.5	2.0000	3.5

parts of the spectrum. The logarithm of the relative energy is plotted against wave length to give the spectral sensibility curve.

The given constant physiological response arbitrarily chosen was a pupillary contraction of 0.5 mm. (cf. Hecht and Pirenne, 1940). In measuring the 0.5 mm. contraction, the starting point was taken as the average size of the completely dark adapted pupil, neglecting the slight differences in these sizes. Since the curves are more or less parallel, a contraction of any amount should give the same results. This has been tried and found to be true.

The spectral sensibility curve determined from the data is shown as the continuous line in figure 3. The position of minimum energy or the maximum visibility is found to be at 510 milli-microns. Visibility falls from this value to low values on either side of the red and violet. This curve is similar to the visibility function for human rod vision as determined by Hecht and Williams (1922) who found the maximum visibility to be at 511 milli-microns, and confirmed by the work of Sloan (1928). The classical dim visibility curve of Hecht and

Williams (1922) is reproduced in figure 3 as the dotted line. It is obvious that both curves agree extremely well, and both represent the spectral sensibility curve of the human eye at low intensities, or rod function. It is definite, from the results, that the rods do play a part in the control of pupillary size. This is in opposition to the view recently advanced by Brown and Page (1939) who have claimed that only the cones influence the movements of the pupil. They arrived at this conclusion merely because of their assumption that the curve of pupillary dilatation in darkness was similar to the curve of subjective dark adaptation. They claimed, from these considerations, that where the pupil showed a rod visibility curve, it resulted "from transmission of excitation from the rods within or outside the macula to optic nerve fibers leading off from cones" which control pupil constriction. This implies that when the cones are stimulated in this manner they cause the pupil to give a false impression of having nervous connections from the rods. This is rather unreasonable, since the cones

Fig. 3. The human dim vision spectral sensitivity curve (continuous line) as determined by pupil diameter measurements. The dotted line represents the same curve from data determined subjectively by Hecht and Williams (1922). The latter curve has been placed slightly below the former so that the maxima of the two may be compared.

would show similar responses once they are stimulated, no matter whether they are stimulated directly or their corresponding optic nerve fibers are stimulated indirectly.

That the rods, in addition to the cones, influence pupil size is borne out by the previous investigations. Both Engelking (1922) and Hess (1909) showed that the pupil in a totally color blind eye constricted and dilated, although more slowly than in the normal eye. Abelsdorff and Feilchenfeld (1904) showed that when the dark adapted eye was stimulated by a large area, the center of which was black, the pupil reflex was evoked. Finally, we have noticed that a distinct pupillary constriction of about 0.5 mm. in the dark adapted eye is obtained by any wave length of light at intensities which are above the visual threshold but which appear colorless to the subject.

The previous attempt of Laurens (1923) to portray spectral visibility curves from measurements of pupillary size does not meet with the requirements demanded by the classical definition of such curves, since instead of measuring

the relative energy to produce the same physiological effect, he determined the relative amount of physiological effect produced by different wave lengths in an equal energy spectrum. The curve in figure 1 is based upon the classical requirements, and when it is compared with other data, it is seen that it not only coincides with the dim visibility curve of the human eye, but also with the absorption spectrum of visual purple. Thus the evidence that the visibility curve as measured in this investigation is a rod function is strengthened.

SUMMARY

A new infrared photographic method for measuring pupil diameter under any condition of light adaptation is described. It is found that the human dim visibility curve obtained by relating pupil diameter to intensity of different monochromatic lights has its maximum at 510 milli-microns. It is similar in all respects to the classical dim visibility curves and to the absorption spectrum of visual purple. It is therefore believed that the pupil size relative to light stimulation is under the control of fibers activated by the rods of the retina, as well as the cones.

We wish to thank Prof. J. M. D. Olmsted for his advice and co-operation during the course of these experiments.

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THE EFFECT OF AN INJURED AREA ON THE ELECTRICAL FIELD OF THE HEART BASED ON EXPERIMENTS WITH MODELS[1]

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Received for publication June 15, 1942

The monophasic injury curve is recorded during ventricular systole by connecting an electrode on an injured area of the exposed ventricle through a galvanometer to another electrode on another part of the body. The records obtained show the development of a relative positivity of the electrode on the injured area with respect to the other electrode during the period of electrical systole. On occasion, a region of relative negativity may develop simultaneously in the immediate neighborhood of the injured area during electrical systole (1). Not only does the injured region become relatively positive to the second electrode during electrical systole, but it becomes relatively negative during electrical diastole (2, 3). Further, the duration of the monophasic injury current (in bipolar leads) is affected by changing the location of the electrode on uninjured regions but not by changing the location of the injured region (and the electrode placed on it) (4). The duration of the monophasic current is also altered by chemicals when they are placed on the region of the uninjured area of electrode but not when placed on the region of the injured area of electrode (4).

We have pointed out (3) that these observations could best be correlated with the classical membrane theory and that the primary source and sink of the currents was in the electrically polarized membrane. At that time we advanced the view that the cell membranes in the injured region could be considered as being in one of four states: *a*, normal resting polarity across the cell membrane during both diastole and systole—the injured area being unresponsive (5); *b*, no polarity across the cell membranes during either diastole or systole; *c*, partial polarity across the cell membranes only during diastole with no polarity during systole—the region being responsive; or *d*, partial polarity across the cell membranes during both diastole and systole—the injured area being unresponsive. Situation *d* fitted the observations of Eyster, Meek et al. (and our own) on the reversal of the potential of the injured area with respect to that existing in uninjured areas.

We felt that these views might further be clarified by model experiments. For this purpose, the situation of the heart in the body can be imitated in principle by two metal rings placed in a large dish filled with saline in such a way that the dish is completely divided into three compartments. The compartment outside the outer ring represents the body field outside the heart; the compartment between the inner and outer rings represents the cell membrane itself separating the charges on its two sides in the normal polarized resting state of the cell; and the compartment inside the inner ring represents the cell interior. The outer and

[1] Aided by the A. D. Nast Fund for Cardiac Research.

inner rings, connected to a source of positive and negative potential represent respectively the positively charged outer surface and the negatively charged inner surface of the resting cell membrane, according to the classical membrane theory. The fact that the geometric pattern of the syncytium of the heart is infinitely complex does not alter the fact that in its normal resting state the heart is not a simple bipole, but the equivalent of an inner negative pole completely surrounded by an outer positive pole, as represented in simple form by our model. In a three dimensional model this would require one sphere within another with similar complete compartment separation. If such a model is employed and the electrical field determined[2], it should give a clue to the potential differences existing in the body as contributed by the heart when the latter is in its resting polarized state.

We found that no potential difference existed in the outside compartment, the potential being identical throughout with that of the outer of the two metal rings[3]. Hence, during the resting polarized state of the heart existing during diastole, the body will have the same potential as the outside of the polarized cell membrane of the heart syncytium[4]. Similarly it was found that the compartment inside the inner ring had everywhere the same potential as the inner ring, that is to say, the potential inside the syncytial cell of the heart is the same as that on the inner surface of the cell membrane when the cell is in the resting polarized state. It is, however, different from that outside the cell. Between the two rings there is a steep potential gradient going from outer to inner ring, the equipotential lines being circles concentric with the rings.

If in such a model the two rings are discharged so that no potential differences exist, the entire electrical field disappears and all parts of the three compartments are at the same potential, the potential of ground. Thus it is obvious that both in the completely polarized state of rest during diastole, and in the completely depolarized state during systole, a unipolar connection will show the

[2] *Method.* The two metal rings, 3″ and 4″ respectively in diameter and 1″ high are set concentrically in a bakelite base and immersed in the center of a large shallow dish filled with approximately 10 per cent saline. The dish was a varnished wooden tray, 21″ x 24″ x 2″. The voltage supply for the rings and the ring segments is obtained from a potentiometer connected across a source of 110 V.A.C. with center point grounded. An exploring electrode is connected through a pair of headphones to the sliding contact of the potentiometer. Thus, using the headphones as a null detector, isopotential lines may be plotted in the field at any desired intervals, the voltage of the exploring electrode with respect to ground being measured with a high resistance voltmeter for each position of the potentiometer slider. The metal rings rested on the bottom of the tray and emerged from the saline bath.

[3] If the outside field is not connected to ground, this will be some value more positive than ground.

[4] In our experiments each ring was connected to the potentiometer by a wire fastened to one point on the circumference. Thus there was a small circumferential potential gradient along each ring, and consequently a slight potential gradient in the inner and outer compartments. Strictly speaking, the potential of the two rings was +5 and −5 respectively only at the point of connection of the lead wires. If, instead of the rings, the electrodes had consisted of two coaxial cones with the lead wires connected to their apices, there would have been no circumferential potential gradient and hence no gradient in the outer and inner compartments.

distant electrode to be at the same potential as the electrode on or close to the heart's surface. Since the potential of the heart's surface is not the same in diastole as in systole, it is obvious that the potential of the distant electrode also is not unchanged between diastole and systole. The distant electrode cannot, therefore, be considered "indifferent" in the sense that it remains at constant potential. The illusion that distance makes the electrical effect of the heart less is not borne out when comparing the completely polarized with the completely depolarized state in the uninjured heart. These observations further demonstrate the need of signifying precisely what reference potential is intended when speaking of relative positivity or relative negativity. Failure to do this, we fear, has led to some of the apparent contradictions in the literature.

Let us try to imitate in the model the state of affairs outlined in *d* above, namely, an injured region in which the cell membrane is only partially polarized during the resting polarized state in diastole of the rest of the ventricles, and remaining in this state because it is unresponsive during the systole of the rest of the ventricles. In order to imitate this condition in the simplified model the two rings were split into a smaller and a larger set of segments (see fig. 1), the smaller segments having a lesser potential difference between them than the larger ones. The former set thus represents the injured region which is only partially polarized, and the latter set, the remaining uninjured cell. The three compartments of the field are now no longer completely separated. As figure 1 shows, the entire outside compartment is still positive but not of uniform potential, the potential being greater near the larger segment than near the smaller, and the potential at more distant points being greater on that side of the field in which the larger segments lie than on the side of the smaller segments, the potential difference between the two sides declining as the outer portions of the field are reached. In short, while the entire external compartment is positive with respect to ground, its potential is not uniform, currents will flow, and the region in the neighborhood of the injured part will be relatively negative to the resting polarized uninjured cell (2, 3). The resting injury current is thus due to the incomplete polarization of the injured region.

The inner compartment, equivalent to the cell interior in the heart, will also show potential differences although all parts will be negative with respect to ground. Thus, the resting injury current flows not only in the external field but within the cell syncytium as well.

During electrical systole, the membrane of the uninjured portion of the syncytial cell becomes depolarized, hence the large segments will no longer be charged, while the small segments will retain their charge as in diastole. The field under these circumstances for the model is illustrated in figure 3. The large segments are removed here because, being made of metal, they are a better conductor than the saline bath and so would distort the field.

There is no longer any separation whatever into compartments. Both negative and positive charges are exposed to the entire external field. While in this particular experiment the zero line and negative field are still within the area formerly covered by the inner compartment, this could be changed by using

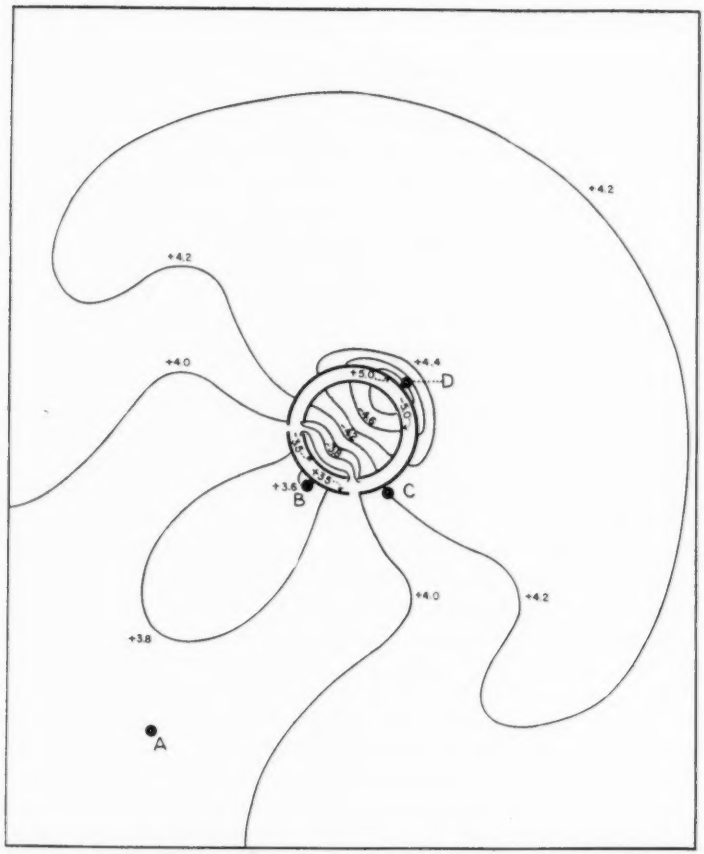

Fig. 1. Distribution of potential around two segmented metal rings, representing the electrical field around an injured heart during the resting polarized state. The pair of smaller segments, representing the partially polarized injured region of the syncytial cell membrane, is connected to a source of smaller voltage than that supplying the larger "completely polarized" uninjured segments. The lines represent equipotential lines are 0.1 volt apart. The figures represent volts. Spot *A* is at a distant point in the field; *B* is near the "injured" region; *C* is near the junction of the "injured" and "uninjured" regions; and *D* is near the "uninjured" region. Discussed in text.

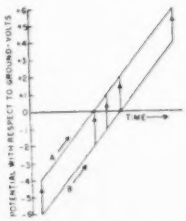

Fig. 2. Diagram illustrating how the potential with respect to ground of two points *A* and *B* in a field may change without affecting the potential difference between the two points. Discussed in text.

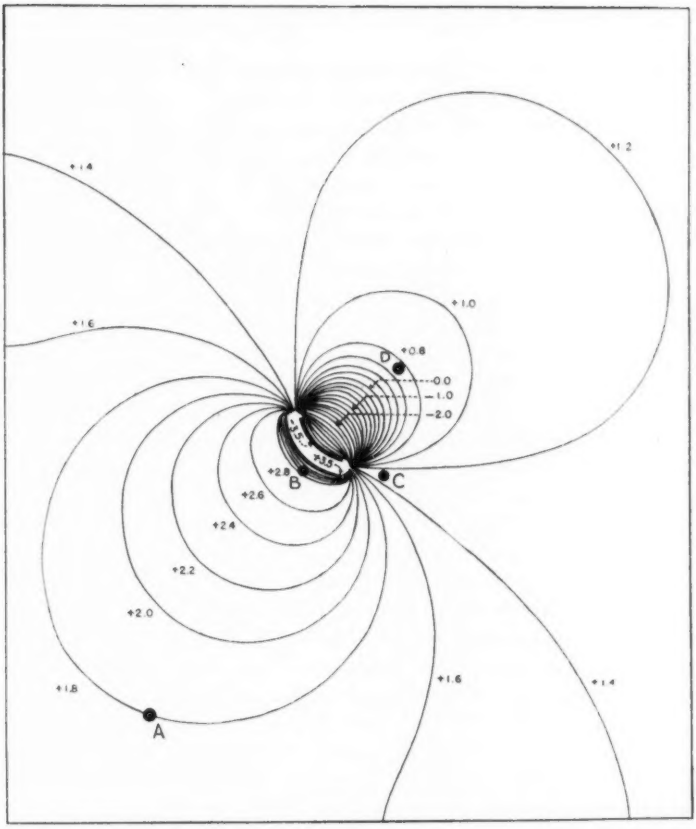

Fig. 3. Distribution of potential around two small metal segments, representing the electrical field of an injured heart during the completely activated state. The large segments of figure 1, now being completely depolarized, have been removed, and the only remaining source of potential is the pair of small segments representing a partially polarized, injured unresponsive region of the cell membrane. Conventions as in figure 1. Discussed in text.

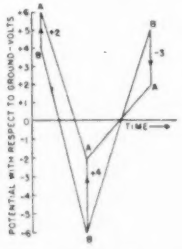

Fig. 4. Diagram illustrating that the direction and magnitude of changes in the potential difference between two points *A* and *B* in a field give no indication of the changes in potential of each point with respect to ground. Discussed in text.

smaller segments for the "injured unresponsive region" and then the negative field would extend outside the confines of the "cell".

In the state of systole as depicted in figure 3, the outer portions of the field are still positive with respect to ground, but, unlike the conditions in diastole in figure 1, now the potential is more positive on the side of the field toward the "area of partially polarized unresponsive injury". Thus an electrode on or near the surface of the "injured region" will be relatively positive to any distant electrode (unipolar lead) or to any electrode on an "uninjured responsive part" of the model (bipolar lead). In short, the activity injury current will be the reverse of the resting injury current (2, 3).

This can be illustrated by comparing the potential difference existing in figures 1 and 3 between distant point A and point B close to the "injured region".

In figure 1, $A = +3.9$ v. and $B = +3.6$ v. so that B is 0.3 v. less than A. In figure 3, however, $A = +1.8$ v. and $B = +3.0$ v. so that B is now 1.2 v. more than A. Thus at rest B over the injured region is relatively negative with respect to the distant electrode A. After activation, however, B over the injured region is relatively positive with respect to the distant electrode A. Yet, the change in potential between figures 1 and 3 has been greater in the distant electrode, 3.0 v. $-$ 1.8 v. or a drop of 1.2 v., than in the electrode B 3.6 v. $-$ 3.0 v. or a drop of 0.6 v. Surely the distant electrode is not "indifferent" in this instance.

Similarly a bipolar connection between B and D, the latter on an uninjured region, will show a similar trend. In figure 1, $B = +3.6$ v. and $D = +4.8$ v. so that B is 1.2 v. less than D. In figure 3, B is +3.0 v. and D +0.7 v. so that B is 2.3 v. more than D. Thus at rest B, over the injured region is relatively negative with respect to D over the uninjured region. After activation, however, B over the injured region is relatively positive with respect to D over the uninjured region. Yet, the change in potential between figures 1 and 3 has been greater over the uninjured, 4.8 v. $-$ 0.7 v. a drop of 4.1 v., than over the injured region, 3.6 v. $-$ 3.0 v. or a drop of 0.6 v.

It is thus obvious that the changes noted by Eyster, Meek et al. (2) which we confirmed (3), do not imply that the injured region is the source for the monophasic action current since the potential in this region is actually little altered during activity of the heart. The results indicate that the change in potential caused by depolarization of the uninjured membrane is responsible for the monophasic action current. This would accord also with the effects we noted on the duration of the monophasic curve.

The observation that a point in the neighborhood of the injured area becomes negative relative to a distant point during systole at the same time that a point on the injured area becomes positive relative to this distant point (1) can also be demonstrated to occur in this model. If, for example, point A in figures 1 and 3 is compared with point C near the smaller "injured" segments, it will be found that unlike B which becomes relatively positive to points A and D during depolarization, spot C becomes relatively negative to these points, viz., in figure 1, $A = +3.9$ v. and $C = +4.2$ v. so that C is 0.3 v. greater than A. At rest, C is positive relative to A while B is negative relative to A. In figure 3, $A = +1.8$ v.

and $C = +1.3$ v., so that C is 0.5 v. less than A. In the activated state, C is relatively negative to A whereas B is relatively positive. Therefore, in the change from the resting to the active state spot C became 0.8 v. relatively negative to A, at the same time that spot B became 1.5 v. relatively positive to A.

This model accounts for all the findings observed in the experiments on the animal. It can therefore be concluded that the monophasic action current in the more complex situation of the cell syncytium is determined primarily by the depolarization and repolarization in the uninjured areas of the heart; the injured area can be conceived as partially depolarized and unresponsive as the result of injury. This state of unchanged polarity in the injured area alters the electrical field during the polarized state of the heart in diastole and the depolarized state in systole from those existing in like periods before injury occurred. Only in this sense can the injury be considered to contribute to the monophasic action current of injury. The actual change in the field, however, between diastole and systole is caused by alterations in the electrical state of the uninjured part of the heart. There is, therefore, no need to accept the contrary interpretation (1, 2) that the monophasic action current is due to potential changes in the injured region. The classical membrane theory adequately accounts for all the facts of the monophasic injury current.

Our results further emphasize one other important fact, namely, that distance from the heart does not exclude the possibility of large changes in potential during the heart cycle. As pointed out in the uninjured cell, the potential in distant regions changes as much as that near the cell between the completely polarized and depolarized states, and in the model with "injury", the distant electrode may show greater changes in potential than regions near the heart.

A similar interpretation of the source of the monophasic action current would apply if the state of the injured cell were either that of *a* or *b* above (p. 779). In the former case no change in potential would occur in the injured area since polarization is present to the same extent during systole and diastole; in the latter case no change in potential would occur in the injured region since depolarization persists during the entire heart cycle. The monophasic injury curve is therefore also due in these cases to the process of depolarization and repolarization of the uninjured part of the cell. However, the reversal of relative potential in the neighborhood of the injured area during activation of the heart would not occur in these circumstances. This would also exclude a partially depolarized state in the injured area during diastole, responsive in systole, condition *c*. However, only in this latter circumstance could the injured region conceivably contribute to the monophasic action potential since its polarity would alter.

It is apparent, therefore, that the whole electrical field of the heart must be examined under varying circumstances in order to elucidate the true origin of the monophasic action current, or of the resting and activity injury currents. This can be aided by model experiments. It cannot be achieved merely by comparing the alterations in potential of two spots in the electrical field in terms relative to each other since this implies that the potential of the spot used as a refer-

ence point is unchanged during the heart cycle with respect to ground potential and this is a fallacious assumption as the present study shows. This may be made clearer by reference to figures 2 and 4. Figure 2 shows that the actual potentials of two points in the field with reference to ground may change without altering the potential difference between them, and figure 4 indicates that there is no parallelism in the magnitude or direction of the actual changes in potential of the two points with respect to ground and the magnitude or direction of the potential difference between them.

These deductions, based on model experiments require confirmation in the animal since conditions in the latter are more complex as regards 1, the relative sizes of the polarized cell membrane, the injured area, and the field 2, the nonhomogeneity in conductivity of the field, and 3, the electrical character of the surface of the body, the field boundary. However, we are inclined to view our results with the model as qualitatively valid because they are in accord with observed changes in the animal.

SUMMARY

The electrical field was explored in models made to represent in simple form the syncytial cell of a normal heart and a heart with an injured, unresponsive area. In the "normal heart" model during electrical diastole the entire external or body field was found to be an equipotential region at the same potential as the external surface of the "polarized cell membrane", i.e., positive with respect to ground. During electrical systole when depolarization was complete the entire field was at ground potential.

In the "injured heart" model during electrical diastole the external field was still positive throughout with respect to ground; however, it was no longer an equipotential region because the "injured region" was only partially polarized. This led to the flow of the resting injury current. During electrical systole the potential distribution in the field was altered due to the depolarization of "uninjured" portions of the "cell membrane", while the "injured region", being unresponsive, retained its partial polarization. This gave rise to the activity injury current. It was shown with these models that distant points in the external field may experience large changes in potential with respect to ground between diastole and systole, and so cannot be considered to be indifferent. Further, the resting injury current and the activity injury current and hence the monophasic curve of injury can be explained in these models on the basis of the classical membrane theory. The activity injury current is shown to be due to the depolarization of uninjured regions and not to any process occurring within the injured area.

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THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. 137—NO. 4

Issued November 1, 1942

BALTIMORE, U. S. A.

1942

Entered as second-class matter, August 18, 1914, at the Post Office in Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

THE AMERICAN JOURNAL OF PHYSIOLOGY

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